# Grape and wine quality of *V. vinifera* L. cv. Cabernet Sauvignon/99R in response to irrigation using winery wastewater

by

**Charl Schoeman**

Thesis presented in partial fulfilment of the requirements for the degree of
**Master of Science in Agriculture**

at
**Stellenbosch University**
Department of Viticulture and Oenology, Faculty of AgriSciences

*Supervisor:* Prof M du Toit
*Co-supervisors:* Prof JJ Hunter
Dr AE Strever

December 2012

# Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: 22 October 2012

# Summary

Grapevine performance and wine quality are influenced by various factors, two of the most important being the availability and quality of irrigation water. In relatively dry countries such as South Africa the conservation and effective use of water is of utmost importance. Expected increases in temperature and decreases in rainfall in the future due to climate change impacts highlights the importance of water conservation. This inspired investigations into possible alternative irrigation water sources and therefore the possibility of vineyard irrigation using winery wastewater is of utmost importance for the sustainability of the wine industry.

Winery wastewater contains higher concentrations of certain elements other than water generally used for vineyard irrigation, the most important differences being Na and K levels. Furthermore, winery wastewater contains larger populations of microorganisms such as yeasts, lactic acid bacteria and acetic acid bacteria, typical associated with wine production. If irrigation using winery wastewater affects the uptake of certain elements or alters grapevine water status, it may affect grapevine growth, juice and wine composition. Furthermore, if juice and wine composition is affected wine composition and sensorial quality may be affected.

Cabernet Sauvignon/99R grapevines, growing in a sandy soil in the Breede River Valley, were subjected to eight irrigation treatments using augmented winery wastewater in addition to irrigation using raw river water as control. The study was carried out during the 2010/11 and 2011/12 seasons. The various wastewater irrigation treatments were made up by augmenting winery wastewater with raw river water to obtain a target chemical oxygen demand (COD) concentration. In this study, the level of COD in the irrigation water is a direct indication of water quality, the two being indirectly proportional. The eight wastewater irrigation treatments ranged from 100 mg/L COD up to 3000 mg/L COD.

The first objective of the study was to determine the effect of irrigation using augmented winery wastewater on grapevine response, with regards to vegetative growth, berry development and berry composition. The various wastewater irrigation treatments did not affect grapevine vegetative growth or reproductive growth, including yield, throughout berry development up to harvest. Berry sugar accumulation and evolution in acid concentrations were also not affected. An increase in berry juice pH was observed with an increase in the level of COD in the augmented winery wastewater only in the second season. The amount of elements, ions and heavy metals in juice was not affected by wastewater irrigation, indicating that there was no absorption by the grapevines. Berry skin thickness, colour and phenolic content as well as yield and its associated components were not affected by irrigation using augmented winery wastewater.

The second objective of the study was to determine the effect of irrigation using augmented winery wastewater on wine microbial and chemical composition, fermentation performance and wine sensorial characteristics. The natural yeast and bacteria flora of juice was not affected by the various wastewater irrigation treatments. In addition, the ability of the inoculated yeast and lactic acid bacteria strains to conduct their respective fermentation processes were not affected. With the exception of total titratable acidity (TTA) and pH, irrigation using augmented winery wastewater did not affect wine chemical composition with regards to basic wine parameters as well as colour, phenolic and tannin composition. Similar to juice, phosphorus and selected ions

were not affected. None of the measured wine sensorial characteristics were affected by irrigation using augmented winery wastewater.

The third objective of the study was to investigate the effect of direct contact between berries and winery wastewater on wine sensorial characteristics. The study focussed on the transference of off-flavours from the wastewater into the wine and the occurrence of off-flavours as a response to contact with winery wastewater. Wine colour and general sensory wine descriptives were not affected by direct contact with winery wastewater. The presence of a winery wastewater-like off-odour and volatile acidity was, however, more detectable in wines made from berries that were in contact with the most concentrated wastewater. Therefore, it may be possible for off-odours to be transferred from the winery wastewater into the wines, or that off-odours are formed as a direct or indirect result of contact with winery wastewater.

Under the given conditions, results obtained in this two seasons of the study suggest that irrigation using augmented winery wastewater does not affect grapevine performance or wine quality substantially. The major impact that was observed was an increase in wine pH and a decreasing trend in TTA. Both these parameters could be rectified by simply adding acid to the wines. Therefore, irrigation using augmented winery wastewater may be considered as a possible future alternative source for vineyard irrigation. It is, however, important to remember that some of the effects of wastewater irrigation may be cumulative and could possibly arise only after several years. Furthermore, different field conditions and cultivars may respond differently.

# Opsomming

Wingerd prestasie en wyngehalte word deur verskeie faktore beïnvloed waarvan twee van die belangrikste die beskikbaarheid en gehalte van besproeiingswater is. In relatiewe droë lande soos Suid Afrika is waterbesparing en die effektiewe benutting van water hulpbronne van uiterste belang. Die verwagte toename in temperatuur en afname in reënval in die toekoms as gevolg van klimaatsveranderinge plaasdieklem op op die belangrikheid van waterbesparing. Dit het navorsing om moontlike alternatiewe vorme van besproeiingswater te ontdek geïnspireer. Na aanleiding van hierdie faktore word daar toenemend gefokus op navorsing oor die moontlikheid om kelder afvalwater as alternatiewe bron van besproeiings water vir wingerde te benut.

Kelder afvalwater bevat hoër konsentrasie van sekere elemente as water wat onder normale omstandighede gebruik word vir die besproeiing van wingerde, die belangrikste verskille was die vlakke van Na en K. Benewens die hoër konsentrasie van sekere elemente bevat kelder afvalwater ook groot populasies van mikroörganismes soos giste, melksuurbakterieë en asynsuurbakterieë, tipies geassosieerd met wynbereiding. Indien besproeiing met kelder afvalwater die opname van sekere elemente of die plant water status beïnvloed, mag wingerd groei, sap en wyn samestelling beïnvloed word. Daar benewens, indien die mikrobiese samestelling van die sap en wyn beïnvloed word sal die samestelling en sensoriese gehalte van die wyn moontlik beïnvloed word.

Cabernet Sauvignon/99R wingerde, geleë in sanderige grond in die Breede Rivier Vallei, is onderwerp aan besproeiing met agt verskillende konsentrasies van verdunde kelder afvalwater, bykomend tot besproeiing met onbehandelde rivier water wat as kontrole gedien het. Hierdie studie is uitgevoer gedurende die 2010/11 en 2011/12 seisoene. Die teiken besproeiings konsentrasies is verkry deur kelder afvalwater met onbehandelde rivier water te verdun tot ‘n sekere chemiese suurstofbehoefte (CSB) konsentrasie bereik is. Die CSB is in hierdie studie ‘n direkte aanduiding van watergehalte, die twee was indirek eweredig tot mekaar. Die agt CSB konsentrasies waarteen die afvalwater besproei is wissel tussen 100 mg/L CSB en 3000 mg/L CSB.

Die eerste doelwit van die studie was om te bepaal wat die effek van besproeiing met verdunde kelder afvalwater op wingerdprestasie, met spesifieke verwysing na vegetatiewe groei, korrelontwikkeling en korrelsamestelling is. Wingerd vegetatiewe en reproduktiewe groei, insluitende opbrengs, is op geen stadium tydens korrelontwikkeling tot en met oes beïnvloed nie. Die laai van suikers gedurende rypwording, sowel as verskuiwings in suurkonsentrasie, is nie deur besproeiing met kelder afvalwater beïnvloed nie. In die tweede seisoen is ‘n toename in sap pH waargeneem soos die CSB konsentrasie van die besproeiings water toegeneem het. Die element, ioon en swaar metaal samestelling van sap was nie beïnvloed deur besproeiing met afvalwater nie wat aandui dat daar geen opname was deur die wingerd nie. Die dikte, kleur en fenoliese samestelling van druifdoppe is ook nie beïnvloed nie.

Die tweede doelwit van die studie was om te bepaal wat die effek van besproeiing met verdunde kelder afvalwater op wyn mikrobiese en chemiese samestelling, fermentasie effektiwiteit en wyn sensoriese eienskappe is. Die verskeie afvalwater besproeiings behandelings het geen effek op die natuurlike gis of bakterieë flora van die sap gehad nie. Die

vermoë van die geïnokuleerde gis en melksuurbakterieë om hul afsonderlike fermentasie prosesse te voltooi is ook nie beïnvloed nie. Met die uitsondering van totale titreerbare suur (TTS) en pH, is die chemiese samestelling van wyne met betrekking tot basiese wyn parameters, kleur, fenole en tanniene nie beïnvloed nie. Soortgelyk aan sap is wyn fosfor en geselekteerde ioon samestelling nie geaffekteer nie. Die sensoriese karakteristieke was eenders vir wyne van alle behandelings.

Die derde doelwit van die studie was om te bepaal wat die effek wat direkte kontak van kelder afvalwater met druiwekorrels op wyn sensoriese eienskappe het. Hierdie studie het gefokus op die oordrag van afgeure vanaf kelder afvalwater na die wyne sowel as die voorkoms van afgeure as ‘n reaksie op kontak met kelder afvalwater. Wyn kleur en algemene sensoriese eienskappe is nie geaffekteer deur kontak tussen druiwe en kelder afvalwater nie. Kelder afvalwater-geassosieerde afgeure en vlugtige suur was meer duidelik waarneembaar in wyne wat gemaak is van druiwe wat in kontak was met die meer gekonsentreerde afvalwater. Dit mag dus moontlik wees dat afgeure vanaf kelder afvalwater oorgedra word na wyne, of dat sekere afgeure gevorm word as ‘n direkte of indirekte reaksie op kontak met kelder afvalwater.

Onder die gegewe toestande oor die twee jaar studie periode het resultate getoon dat besproeiing met verdunde kelder afvalwater nie wingerdprestasie en wyn gehalte aansienlik beïnvloed nie. Die grootste impak wat afvalwater besproeiing gehad het, was om ‘n toename in wyn pH en ‘n tendens tot afname in TTS te veroorsaak. Deur eenvoudig suur by die wyn te voeg kan albei hierdie probleme reg gestel word. Op grond van hierdie bevindings kan besproeiing met verdunde kelder afvalwater moontlik as toekomstige bron vir addisionele wingerdbesproeiing dien. Dit is egter belangrik om te onthou dat die effekte van besproeiing met kelder afvalwater mootlik kumulatief kan wees en dat probleme moontlik eers na etlike jare na vore kan kom. Ander kultivars en veldkondisies mag ook lei tot ander resultate.

This thesis is dedicated to

My parents

---

Hierdie tesis is opgedra aan

My ouers

# Biographical sketch

Charl Schoeman was born on 24 February 1988 in Paarl and matriculated at Huguenot High School (Wellington) in 2006. In 2007 he enrolled at the Stellenbosch University for a BScAgric in Viticulture and Oenology and obtained his degree in 2010. In 2011, he enrolled at the same University for a MScAgric in Oenology.

# Acknowledgements

I wish to express my sincere gratitude and appreciation to the following persons and institutions:

- **My Heavenly Father** who has given me the grace, patience, strength, endurance and capability to complete this study;

- **Prof M du Toit,** (Institute for Wine Biotechnology, Department of Viticulture and Oenology, Stellenbosch University) for her encouragement, inputs, advice, assistance and her friendly, helpful attitude during laboratory work and writing of the thesis;

- **Prof JJ Hunter,** (ARC Infruitec-Nietvoorbij, Stellenbosch) for his encouragement, inputs, advice and willingness to always assist in field and laboratory work and writing of the thesis;

- **Dr AE Strever,** (Department of Viticulture and Oenology, Stellenbosch University) for his inputs, advice and assistance in writing of the thesis;

- **Dr PA Myburgh, C Howell, Vink Lategan and RA Stolk,** (ARC Infruitec-Nietvoorbij, Stellenbosch) for their friendship, enthusiasm for the project, advice and inputs throughout field and laboratory work and writing of the thesis;

- **Prof M Kidd,** (Stellenbosch University) for help with processing and understanding the statistical data;

- **Cellar staff,** (ARC Infruitec-Nietvoorbij, Stellenbosch) for support, assistance and willingness to help in the cellar.

- **The Water Research Commision, Winetech and ARC Infruitec-Nietvoorbij** for co-funding the project and my MSc studies;

- **The DVO and IWBT, (**Stellenbosch University) for partial bursary funding and giving me the opportunity to do my MSc study;

- **Management at Goudini Winery,** for allowing us to conduct the field trial on their premises;

- **My parents and brother,** Louis, Carmen and Michael, for their constant support, encouragement, love and for never failing to believe in me; and

- **Amy Engelbrecht,** for her understanding, love, support and encouragement throughout the study.

# Preface

This thesis is presented as a compilation of 6 chapters. Each chapter is introduced separately and is written according to the style of the South African Journal of Enology and Viticulture.

| | |
|---|---|
| **Chapter 1** | **General Introduction and Project Aims** |
| **Chapter 2** | **Literature review**<br>Wastewater: Use in Agriculture |
| **Chapter 3** | **Research results**<br>The effect of irrigation using winery wastewater on grapevine growth, berry development and berry composition of Cabernet Sauvignon/99R in the Breede River Valley |
| **Chapter 4** | **Research results**<br>The effect of irrigation using winery wastewater on juice and wine microbial flora, wine chemical composition and sensorial characteristics of Cabernet Sauvignon/99R in the Breede River Valley |
| **Chapter 5** | **Research results**<br>Effect of direct contact between berries and winery wastewater on wine sensorial characteristics |
| **Chapter 6** | **General Discussion and Conclusions** |
| **Addendum A** | **Microbial flora of irrigation water and must** |

# Table of Contents

# Chapter 1

## Introduction and project aims

# 1. INTRODUCTION AND PROJECT AIMS

## 1.1 INTRODUCTION

Water is one of the most important resources required for plant growth and crop production. South Africa is a relatively dry country, receiving an average annual rainfall of 450 mm and having a high evaporation rate (Department of water affairs and forestry, 2004). Thus, South Africa receives barely more than half the mean annual world rainfall of 860 mm (Department of water affairs and forestry, 2004). In South Africa, 95% of the 101 325 hectares of wine grape vineyards are planted in the Western Cape, receiving a mean annual rainfall of 348 mm (Cupido & Isaacs, 2009; Department of water affairs and forestry, 2004). It is estimated that the mean temperature in South Africa will increase in the range of 1 ˚C to 3 ˚C by the middle of the 21$^{st}$ century, as a result of global climate change (Department of Environmental Affairs and Tourism, 2004). Furthermore, a broad reduction in rainfall of between 5% and 10% is expected for the summer rainfall region, while a marginal increase in rainfall is expected in the early winter for the winter rainfall region (Department of Environmental Affairs and Tourism, 2004). The increase in temperature and decrease in rainfall will lead to increased pressure on available water resources. The importance of water conservation and judicious water use is therefore of utmost importance, especially in the agricultural sector. Water for irrigation accounted for 62% of the 12,496 million m$^3$ total water withdrawal of South Africa in the year 2000 (Food and Agriculture Organization, 2008). Water re-use may be an effective means of relieving some of the pressure on water resources. For this reason, increasing research focus is being placed on the use of winery wastewater as alternative source for vineyard irrigation (Laurenson *et al.,* 2010).

The South African wine industry generates more than 1000 million litres of wastewater annually (Sheridan, 2007). All of this wastewater needs to be disposed of in accordance with government legislation and the means of disposal must be authorised by the Department of Water Affairs and Forestry (DWAF) (National Water Act, 1998). More than 95% of existing wineries in South Africa dispose their winery wastewater onto land (Van Schoor, 2005). Care must be taken that wastewater disposal does not harm the crop or soil. Winery wastewater contains high levels of organic matter, and thus high chemical oxygen demand levels (COD). The COD is a measure of the total organic content in water in terms of the amount of oxygen needed for its total breakdown *via* oxidation. Winery wastewater contains higher concentrations of certain elements, the most important being sodium (Na) and potassium (K) (Mulidzi *et al.,* 2009; Sheridan *et al.,* 2011). The application of nutrient rich wastewater may therefore increase the concentrations of these nutrients in plant tissue and affect plant growth and fruit composition

(McCarthy, 1981; Neilsen *et al.,* 1989; Laurenson *et al.,* 2010; Stevens *et al.,* 2011). Moreover, irrigating with high strength untreated wastewater can cause damage to even the toughest of crops (Van Schoor, 2005). Irrigation with raw and diluted winery wastewater was found to inhibit vegetative growth of barley, millet, lucerne and phalaris (Mosse *et al*., 2010).

An increase in juice Na and K results in a decrease in berry malic and tartaric acids and an associated increase in juice and wine pH (Somers, 1975; Iland & Coombe, 1988 Mpelasoka *et al.,* 2003; Stevens *et al.,* 2011). Furthermore, high pH wines generally taste flat and red wines with high pH values have an undesirable brownish hue (Gladstones 1992; Rühl 1989). High pH wines are also more prone to microbial spoilage. Another negative consequence of increased juice Na levels is the possibility of an increase in undesirable phenolic compounds in the resulting wine (White, 2003). An increase in wine sodium chloride (NaCl) concentration, due to saline soil conditions, has been found to extend the duration of alcoholic fermentation using S. *cerevisiae* while leading to the formation of elevated concentrations of acetic acid and glycerol (Donkin *et al.,* 2010).

Winery wastewater irrigation is known to cause an increase in soil salinity (Australian Environmental Protection Authority, 2004). Due to its high sodium adsorption ratio (SAR), electrical conductivity (EC) and organic content, winery wastewater irrigation may also cause soil sodicity, chemical contamination, waterlogging and anaerobiosis, loss of soil structure and an increased susceptibility to erosion. The SAR is the amount of Na present in the water, relative to calcium (Ca) and magnesium (Mg). The EC is an indication of the amount of dissolved salts in the water. If the soil is detrimentally affected it is certain that the grapevine will be influenced in some way or another (Van Schoor, 2005). Additional effects of winery wastewater irrigation on crops require further investigation.

The above mentioned facts indicate that winery wastewater flowing out of the cellar more often than not needs treatment of some sorts in order to be of acceptable quality to irrigate onto land (Ryder, 1995; Van Schoor, 2004). The treatment of winery wastewater is however not necessarily a sustainable option as it is expensive and associated with high energy use and emission of greenhouse gasses which may have a major impact on the carbon footprint in wine-producing regions (Rosso *et al.,* 2009). During aerobic wastewater treatment organic pollutants are oxidized to form mainly $CO_2$ and water (Seabloom & Buchanan, 2004). On the other hand, anaerobic wastewater treatment entails the conversion of organic pollutants into, along with other compounds, $CO_2$ and methane ($CH_4$) (McCarty, 1964). Methane ($CH_4$) and $CO_2$ are two of the three most important and harmful greenhouse gasses (Department of Environmental Affairs and Tourism, 2004). Furthermore, high energy usage may contribute to even greater electricity shortages than already experienced in South Africa.

Due to low and erratic summer rainfall, most vineyards in the Western Cape require irrigation. It would be ideal if a sustainable use of winery wastewater could be achieved by implementing supplementary wastewater irrigation or by adding the wastewater to existing water resources for irrigation purposes. The dilution of winery wastewater prior to irrigation has been found effective in some cases, but in others it was found to be an inadequate means of mitigating the phytotoxic effects of winery wastewater (Mosse *et al.,* 2010). Vineyard irrigation with reclaimed winery wastewater has been successfully practised in California for nearly fifty years (Ryder, 1995). Supplementary winery wastewater irrigation can even increase vineyard harvest yield (Ryder, 1995). However, it is unknown how much wastewater a vineyard could tolerate before the soil biota are affected negatively (Kumar *et al.,* 2009). Nonetheless, irrigation of recycled water is gaining increasing acceptance in Australia and becoming a recognized sustainable water resource (Boland *et al.,* 2006; Radcliffe, 2007). Furthermore, the DWAF in South Africa supports the judicious and beneficial irrigation of crops with treated winery wastewater (Van Schoor, 2005). However, the impacts of vineyard irrigation with winery wastewater have not been studied comprehensively and further research is required before vineyard irrigation with augmented winery wastewater can be established as standard practice. The augmentation of winery wastewater, referring to the dilution of winery wastewater with raw water, for irrigation purposes may even become necessary or obligatory in the near future if it can be proven that the augmented water does not affect crops and soil in a negative manner (henceforth, “raw water” will refer to water coming directly from a river or borehole without prior treatment).

## 1.2 PROJECT AIMS

This project formed part of a larger research programme (Project nr WW19/14), co-funded by the Water Research Commission, Agricultural Research Council Infruitec-Nietvoorbij and Winetech. The aim of the project is to investigate the future use of winery wastewater as an additional water source for vineyard irrigation in South Africa. The primary goal of the programme is to investigate the effects of irrigation with winery wastewater, augmented to different levels of chemical oxygen demand (COD) with raw irrigation water, on soil chemical and physical properties, grapevine response, juice and wine composition, and sensorial wine quality in the Breede River Valley. Soil analysis was done as part of a separate, but linked study and will thus not be included in this thesis.

The specific objectives of this study were:

- To determine the effect of irrigation with augmented winery wastewater on grapevine response, such as vegetative growth, berry development and berry composition;
- To assess the effect of irrigation with augmented winery wastewater on wine chemical and microbial composition and fermentation performance;

- To perform sensory evaluations on wines made from augmented winery wastewater irrigated grapevines, focusing on the occurrence of wastewater-associated off-flavours; and
- To evaluate the effect of raw and augmented winery wastewater, sprayed directly onto grapevine bunches, on wine sensory quality by means of aroma evaluation.

## 1.3 LITERATURE CITED


Australian Environmental Protection Authority, 2004. Guidelines for wineries and distilleries. January, 2004. (www.apal.com.au/site/DefaultSite/filesystem/.../EPAguidelines.pdf).

Boland, A., Hamilton, A., Stevens, D. & Ziehrl, A., 2006. Opportunities for reclaimed water use in Australian agriculture. In: Stevens, D.P. (ed). Growing crops with reclaimed wastewater, CSIRO Publishing, Melbourne. pp. 81-90.

Cupido, J. & Isaacs, N., 2009. Statistics of wine-grape vines as on 30 November 2008. South African Wine Industry Information & Systems. P.O. Box 238, Paarl, 7620, South Africa. (http://www.sawis.co.za).

Department of Environmental Affairs and Tourism, 2004. A national climate change response strategy for South Africa. *(*unfccc.int/files/meetings/seminar/.../pdf/sem_sup3_south_africa.pdf).

Department of Water Affairs and Forestry, 2004. First Edition, September 2004. Department of Water Affairs and Forestry, Pretoria, South Africa. *(*www.dwaf.gov.za/Documents/Policies/NWRS/Default.htm).

Donkin, R., Robinson, S., Sumby, K., Harris, V., McBryde, C. & Jiranek, V., 2010. Sodium chloride in Australian grape juice and its effect on alcoholic and malolactic fermentation. Am. J. Enol. Vitic. 61, 392-400.

Food and Agriculture Organization, 2008. The encyclopedia of earth. Water profile of South Africa. *(*www.eoearth.org/article/Water_profile_of_South_Africa).

Gladstones, J., 1992. Viticulture and environment: A study of the effects of environment on grape-growing and wine qualities, with emphasis on present and future areas for growing wine grapes in Australia. Winetitles, Adelaide.

Iland, P.G. & Coombe, B.G., 1988. Malate, tartrate, potassium and sodium in flesh and skin of Shiraz grapes during ripening: concentration and compartementation. Am. J. Enol. Vitic. 39, 71-76.

Kumar, A., Arienzo, M., Quayle, W., Christen, E., Grocke, S., Fattore, A., Doan, H., Gonzago, D., Zandonna, R., Bartrop, K., Smith, L., Correl, R. & Kookana, R., 2009. Developing a systematic approach to winery wastewater management. CSIRO Land and Water Science Report. pp. 1-131.

Laurenson, S., Bolan, N., Smith, E. & McCarthy, M., 2010. Winery wastewater irrigation: effects of sodium and potassium on soil structure. CRC CARE Technical Report 19, 1-25. (http://www.crccare.com/publications/downloads/CRC-CARE-Tech-Report-19.pdf).

McCarthy, M.G., 1981. Irrigation of grapevines with sewage effluent. I. Effects on yield and petiole composition. Am. J. Enol. Vitic. 32, 189-196.

McCarty, P.L., 1964. Anaerobic waste treatment fundamentals. Chemistry and Microbiology, Part I. Public Works 95, 91-112.

Mosse, K.P.M., Patti, A.F., Christen, E.W. & Cavagnaro, T.R., 2010. Winery wastewater inhibits seed germination and vegetative growth of common crop species. J. Hazard. Mater. 180, 63-70.

Mpelasoka, B.S., Schachtman, D.R., Treeby, M.T. & Thomas, M.R., 2003. A review of potassium nutrition in grapevines with special emphasis on berry accumulation. Aust. J. Grape Wine Res. 9, 154-168.

Mulidzi, A.R., Wooldridge, J., Laker, M.C. & Van Schoor, L., 2009. Composition of effluents from wineries in the Western and Northern Cape Provinces I. Seasonal variations and differences between wineries. Wineland, January. pp. 88-91.

National Water Act, 1998. (Act 36 of 1998) Section 21, Government Notice 1091, Government Gazette 19182, 26. August 1998, South Africa.

Neilsen, G., Stevenson, D. & Fitzpatrick, J., 1989. The effect of municipal wastewater irrigation and rate of N fertilization on petiole composition, yield and quality of Okanagan Riesling grapes. Can. J. Plant Sci. 69, 1285-1294.

Radcliffe, J.C., 2007. Advances in water recycling in Australia 2004-2007. In: Khan, S.J., Stuetz, R.M. & Anderson, J.M. (eds). Water Reuse and Recycling. Proc. 3rd AWA Water Reuse and Recycling Conf,, 2007, Sydney, Australia. pp. 387-406.

Rosso, D. & Bolzonella, D., 2009. Carbon footprint of aerobic biological treatment of winery wastewater. Water Sci. Technol. 60, 1185-1189.

Rühl, E.H., 1989. Effect of potassium and nitrogen supply on the distribution of minerals and organic-acids and the composition of grape juice of Sultana vines. Aust. J. Exp. Agr. 29, 133-137.

Ryder, R.A., 1995. Aerobic pond treatment of winery wastewater for vineyard irrigation by drip and spray system in California. Rev. Fr. Oenol. 152, 22-24.

Seabloom, R.W. & Buchanan, J.R., 2005. Aerobic treatment of wastewater and aerobic treatment units. In: Gross, M.A. & Deal, N.E. (eds). University curriculum development for decentralized wastewater management. University of Arkansas, Fayetteville, Arkansas. pp. 1-22.

Sheridan, C., 2007. Constructed wetlands for the primary treatment of winery effluent. MSc thesis, University of Cape Town, Private Bag X3, 7701 Rondebosch, South Africa.

Sheridan, C.M., Glasser, D., Hildebrandt, D., Petersen, J. & Rohwers, J., 2011. An annual and seasonal characterisation of winery effluent in South Africa. S. Afr. J. Enol. Vitic. 32, 1-8.

Somers, T.C., 1975. In search of quality for red wines. Food Technol. Aust. 27, 49-56.

Stevens, R.M., Harvey, G. & Partington, D.L., 2011. Irrigation of grapevines with saline water at different growth stages: effect on leaf, wood and juice composition. Aust. J. Grape Wine Res. 17, 239-248.

Van Schoor, L.H., 2004. A prototype ISO 14001 environmental management system for wine cellars. MSc thesis, Stellenbosch University, Private Bag X1, 7602 Matieland (Stellenbosch), South Africa.

Van Schoor, L.H., 2005. Guidelines for management of wastewater and solid waste at existing wineries. Winetech, South Africa. (http://www.winetech.co.za/index.php).

White, R.E., 2003. Soils for Fine Wines, Oxford University Press, New York.

# Chapter 2

## Literature review

### Wastewater: Use in Agriculture

# 2. LITERATURE REVIEW

## 2.1 INTRODUCTION

The wine industry is an important contributing sector to the South African economy, especially in the Western Cape. The country harvested a total of 1.013 million tons of grapes in 2011, 82.4% of which was used for wine making (WOSA, 2011). These large volumes of wine result in the production of large volumes of winery wastewater. The annual amount of wastewater produced by the South African wine industry is greater than 1000 million litres, representing a considerable threat to the environment (Sheridan, 2007). One billion litres of wastewater is probably a very conservative estimation, as Van Schoor and Rossouw (2004) reported that 2-14 litres of wastewater is produced for every one litre of wine.

Winery wastewater mainly originates from cleaning processes, solid waste (skins, stems, pips and lees), the use of filter material and filter aids, as well as the use of settling and fining agents (Van Schoor, 2000, 2001a; Chapman *et al*., 2001). The primary processes that contribute to the total volume of winery wastewater throughout the year are displayed in Table 2.1 (Chapman *et al.*, 2001; Winetech, 2003). Cleaning processes, being responsible for the majority of wastewater generated (Table 2.1), need to be performed judiciously to ensure that the quality of winery wastewater is of acceptable standard. Due to the variation in composition of different cleaning agents, they have varying impacts on wastewater composition and quality. Generally it is recommended that products which contain sodium, cause high COD concentrations and other salt containing products are used to a minimum in the winery (Van Schoor, 2005).

In this study, the level of COD in the irrigation water is a direct indication of water quality, the two being indirectly proportional. By using “caustic” (NaOH) and other Na-based cleaning agents, the Na concentration is increased in the wastewater being generated, resulting in a higher SAR as well as higher EC for the water. Irrigating Na-rich wastewater may lead to a decrease in the osmotic potential of the soil solutions which impedes with plant water uptake (Walker, 1994). In addition, irrigation using Na-rich water may lead to soil structure degradation (Laurenson *et al.,* 2010). The use of NaOH should, therefore, be replaced by KOH. Using phosphoric acid to flush out tanks, instead of the more commonly used citric acid, will reduce the COD concentration of the winery wastewater being produced (Glaetzer, 2000). Phosphoric acid has a lesser contribution than citric acid to the COD because it is an inorganic acid whereas citric acid is an organic acid. In addition, products that are based on similar compounds may contain varying amounts of harmful elements, such as Na. Therefore, material safety data sheets should be requested before ordering any products to ensure they are as environment friendly as possible. The composition and volume of the wastewater changes throughout the

year and are largely dependent on cellar activity. Winery wastewater contains higher concentrations of certain elements than water normally used for crop irrigation, while it also contains elevated levels of microorganisms (Jourjon *et al*., 2005; Mulidzi *et al.,* 2009; Sheridan *et al.,* 2011). As a result of the possible high Na and K content in winery wastewater, it may have a high EC and SAR.

**Table 2.1** Major processes related to winery wastewater generation and their associated contribution to wastewater quality and quantity.

| Winery operation | Contribution to total wastewater quantity | Contribution to wastewater quality | Effect on legal wastewater quality parameters |
|---|---|---|---|
| Cleaning water | | | |
| Alkali washing (removal of K-bitartrate) and neutralization | Up to 33% | Increase in Na, K, COD and pH<br>Decrease in pH | Increase in EC*, SAR*, COD*<br>Variation in pH |
| Rinse water (tanks, floors, transfer lines, bottles, barrels, etc) | Up to 43% | Increase in Na, P, Cl and COD | Increase in EC, SAR, COD<br>Variation in pH |
| Process water | | | |
| Filtration with filter aid | Up to 15% | Various contaminants | Increase COD and EC |
| Acidification and stabilization of wine | Up to 3% | $H_2SO_4$ or NaCl | Increase COD and EC<br>Decrease in pH |
| Cooling tower waste | Up to 6% | Various salts | Increase COD and EC |
| Other sources | | | |
| Laboratory practises | Up to 5-10% | Various salts, variation in pH, etc. | Increase COD and EC |

* EC = Electrical conductivity; SAR = Sodium adsorption ratio; COD = Chemical oxygen demand

(Chapman *et al.*, 2001; Winetech, 2003)

The increase in wine production over the last decade has increased the impact of the South African wine industry on natural resources such as water, soil and vegetation (Van Schoor, 2005). If untreated winery wastewater is discharged into water bodies or onto land areas it may have a detrimental effect on the environment (Caballero *et al.,* 2010). Furthermore, it may lead to oxygen depletion within aquatic environments which will have an impact on the functioning of the ecosystem. Exposure to wastewater can also lead to salination and eutrophication of water sources (Van Schoor, 2005). Effects on soil include an increase in soil sodicity and/or salinity, chemical contamination, waterlogging and anaerobiosis, degradation of soil structure, as well as

an increase in susceptibility to erosion (Chapman *et al.*, 2001). Furthermore, salinity causes a decrease in the osmotic potential of the soil solution, impeding plant water uptake and resulting in a decrease in plant transpiration, photosynthesis and growth (Walker *et al.,* 1981; Munns and Termaat, 1986; Shannon and Grieve, 1999). Irrigating with nutrient rich and saline wastewater may lead to alterations within plant tissue composition, fruit quality and growth (Laurenson *et al.,* 2010; Stevens *et al.,* 2011). It is therefore important that winery wastewater be disposed of in an effective manner in accordance with government policy. Furthermore, increasing pressure on available resources has led to a tightening of environmental legislation regarding wastewater disposal (National Water Act, 1998; Department of Water Affairs and Forestry, 2004).

This literature review will summarise key aspects associated with the use of winery wastewater for irrigation:

- The chemical composition of winery wastewater
- The microbial composition of winery wastewater
- The effects of various types of wastewater irrigation on different crops
- Government legislation regarding the disposal of winery wastewater
- The effectiveness of wastewater disposal in the South African wine industry.

## 2.2 CHEMICAL COMPOSITION OF WINERY WASTEWATER

Winery wastewater composition is highly variable and changes constantly throughout the year depending on which cellar activities are being performed (Chapman, 1996; Van Schoor, 2005). Moreover, wastewater composition is largely variable between different cellars. The most important quality parameters are pH, sodium adsorption ratio (SAR), chemical oxygen demand (COD) and electrical conductivity (EC). South African wineries display considerable variation in terms of these parameters as research done by Van Schoor (2004) indicates (Table 2.2). Winery wastewater usually contains high concentrations of organic material, mostly sugars and organic acids (tartaric, acetic and propionic acids), esters and polyphenols (Malandra *et al*., 2003). The wastewater contains much more organic matter during harvesting and winemaking than during the bottling period (Racault and Lenoir*,* 1994; Jourjon *et al*., 2001). Furthermore, winery wastewater contains higher concentrations of certain nutrients than typical water used for irrigation (Mulidzi *et al.,* 2009a; Sheridan *et al.,* 2011). The dominant metallic species in winery wastewater are Na, K, calcium (Ca), magnesium (Mg) and iron (Fe), the most important of these being Na and K (Sheridan *et al.,* 2011). Furthermore, zinc (Zn), copper (Cu), lead (Pb) and manganese (Mn) are present at low concentrations, while chromium (Cr), boron (B) and arsenic (As) are not present at detectable concentrations. The reason for the high Na and K concentrations in winery wastewater when compared to typical sources of irrigation water is

primarily due to the use of NaOH and KOH, as well is tartrate crystals washed from tanks after cold stabalisation (Van Schoor, 2005; Kumar *et al.*, 2009). Table 2.3 indicates the variability in chemical composition of winery wastewater composition at a typical cooperative cellar in South Africa.

**Table 2.2** The pH, sodium adsorption ratio, chemical oxygen demand and electrical conductivity ranges in untreated wastewater from South African wineries (Van Schoor, 2004).

| Parameter and unit | Minimum | Maximum | Average |
|---|---|---|---|
| pH | 2.7 | 7.9 | 5.1 |
| SAR | 0.3 | 29 | 5.2 |
| COD (mg/L) | 15 | 70683 | 7433 |
| EC (mS/m) | 16 | 2570 | 279 |

## 2.3 MICROBIAL COMPOSITION OF WINERY WASTEWATER

Winery wastewater contains high numbers of microorganisms, ranging from $10^5$ to $10^8$ colony forming units per millilitre (cfu/mL) (Jourjon *et al*., 2005). Malandra *et al.* (2003) reported that yeast cells were present at 4 x $10^4$ cfu/mL and bacteria were present at 1.64 x $10^6$ cfu/mL in winery wastewater sampled in the Stellenbosch region, South Africa. Seven yeast species and eight bacterial species were identified. The dominant yeast species were *Saccharomyces cerevisiae, Candida intermedia, Hanseniaspora uvarum* and *Pichia membranaefaciens* which are all yeast species forming part of the natural microbial flora of grapes and/or water. Coetzee *et al*. (2004) reported the exact same yeast species to be dominant in a rotating biological contactor during the treatment of winery wastewater. On the other hand, Petruccioli *et al.* (2000) reported that the microbial composition of winery wastewater during effluent bio-treatment predominantly belonged to the genera *Pseudomonas* and *Bacillus* while *Saccharomyces cerevisiae* was always present in their winery wastewater. Similar results with regards to yeasts and bacteria were reported by Eusébio *et al*. (2005).

When the microbial flora in the liquid and biofilm of an aerobic jet-looped activated sludge reactor used for the degradation of winery wastewater was evaluated, yeasts and filamentous cells represented the dominant microflora (Malandra *et al.,* 2003). Furthermore, *Trichosporon capitatum* and *Geotrichum peniculatum* were found to be present in their hyphal form. These organisms formed communities with microbes such as *Saccharomyces cerevisiae*, *Pseudomonas* and metazoan microbes. In contrast to these findings Eusébio *et al*. (2005) found no filamentous fungi to be present inside a bioreactor used for the treatment of winery wastewater.

**Table 2.3** Variation in winery wastewater composition between September and February at a typical cooperative cellar in South Africa.

| Parameter and unit | Units | Date | |
|---|---|---|---|
| | | 14-Sep-11 | 14-Feb-12 |
| pH | | 5.5 | 3.6 |
| TDS | mg/L | 1534 | 557 |
| SAR | | 1.12 | 0.97 |
| COD | mg/L | 4390 | 14440 |
| EC | mS/m | 243.0 | 74.3 |
| Na | mg/L | 229.7 | 44.4 |
| K | mg/L | 357.8 | 120.9 |
| Ca | mg/L | 21.1 | 27.2 |
| Mg | mg/L | 4.80 | 10.06 |
| Fe | mg/L | 4.22 | 3.40 |
| Cl | mg/L | 57.4 | 46.8 |
| $CO_3$ | mg/L | 0 | 0 |
| $HCO_3$ | mg/L | 1232.5 | 0 |
| $SO_4$ | mg/L | 397.0 | 53.6 |
| B | mg/L | 0.21 | 0.61 |
| Mn | mg/L | 0.20 | 0.14 |
| Cu | mg/L | 0.140 | 0.008 |
| Zn | mg/L | 0.360 | 0.148 |
| P | mg/L | 7.25 | 1.68 |
| F | mg/L | 0 | 0.337 |
| Cr | mg/L | 0.039 | 0.005 |
| Cd | mg/L | 0 | 0.001 |
| As | mg/L | 0 | 0 |
| Pb | mg/L | 0.009 | 0.005 |
| Hg | mg/L | 0 | 0 |

*TDS = Total dissolved solids

At the beginning of harvest a high quantity of lactic acid bacteria and yeasts are present in the produced winery wastewater whereas very small quantities of aerobic bacteria are observed (Jourjon *et al.,* 2005). However, at the end of harvest the aerobic flora, including acetic acid bacteria are dominant. Thus, Jourjon *et al*. (2005) reported the microbial composition of winery wastewater to be tied closely to the time of year and winery activity where some microorganisms are favoured during certain periods while others during other periods.

Winery wastewater generally contains small quantities of faecal bacteria and therefore represents a minor sanitary risk (Jourjon *et al.,* 2005). A study by Moncault (2003) estimated *Enterococcus* and *Escherichia coli* counts in winery wastewater at between 1 and 10 cfu/mL and between 10 and 100 cfu/mL, respectively.

The presence of certain microorganisms is closely correlated to certain physical-chemical parameters in wastewater, such as COD. These physical-chemical parameters are however difficult to use to estimate microbial populations present in the wastewater (Jourjon *et al.,* 2005).

## 2.4 EFFECT OF WASTEWATER IRRIGATION ON CROP PRODUCTION AND FRUIT QUALITY

Due to a shortage of studies focusing on the effects of winery wastewater irrigation on crops, this section will incorporate the effects of various types of wastewater irrigation on crop performance and fruit quality. Wastewater is water that has been used for washing, flushing, or in a manufacturing process, and therefore contains waste products. Wastewater originating from different sources has different compositions. Wastewater is usually a nutrient rich water supply, containing higher amounts of certain nutrients than raw irrigation water (Neilsen *et al*., 1989a; Lapeña *et al*., 1995; Mulidzi *et al.,* 2009). Furthermore, wastewater from different origins often has certain similar characteristics such as high salt concentrations. The soluble salt concentration of winery wastewater for instance is similar to that of municipal wastewater, except for higher K levels in winery wastewater due to the use of K-based products for washing (Laurenson *et al.,* 2010). Irrigating with these nutrient rich water sources may reduce fertilization costs, but may also lead to soil structure degradation or alterations in plant tissue composition and/or fruit quality (Neilsen *et al*., 1989b, 1991; Lapeña *et al*., 1995; Laurenson *et al.,* 2010; Stevens *et al.,* 2011). Wastewater is often treated before irrigation to minimize negative impacts of the wastewater on soil or crops or to comply with government legislation (Van Schoor, 2005). This section will be looking at the effects of irrigation with various types of wastewater on seed germination, plant nutrient status and on crop growth, yield and fruit quality.

### 2.4.1 Effect on seed germination

Wastewater irrigation has detrimental effects on seed germination, resulting in an increased time to germination. In a study by Mosse *et al.* (2010) on barley (*Hordeum vulgare*), millet (*Pennisetum glaucum*), lucerne (*Medicago sativa*) and phalaris, increasing concentrations of winery wastewater caused an increased time to germination in all species except for barley. The germination index decreased for all species irrigated with winery wastewater, the higher the concentration of augmented winery wastewater (% of winery wastewater in total volume of water) the larger the decrease (Mosse *et al*., 2010). Similar results were obtained by irrigating monosodium glutamate wastewater on tomato, Chinese cabbage and wheat (Liu *et al*., 2006).

Wastewater irrigation of certain origins may thus inhibit seed germination and decrease the germination index of certain crop species. This may have a negative impact on crop production and economic aspects of production. Due to the chemical composition of wastewater, it may increase the levels of certain elements such as Na and K in the soil (Laurenson *et al.,* 2010). When wastewater irrigation is applied on crops such as grapevines, an interception crop with the purpose of extracting some of these elements will be advantageous. If wastewater irrigation inhibits the germination of these cover crops it would lessen the degree to which they remove excessive amounts of elements such as Na and K. Therefore, if wastewater irrigation is applied on seed sown crops, care should be taken to ensure that wastewater composition is of such a nature that it will not inhibit seed germination.

### 2.4.2 Effect on soil osmotic potential and plant water uptake

Irrigation using Na-rich water, such as winery wastewater, may lead to the development of saline soil conditions (Van Schoor, 2005). Soil salinity is one of the biggest problems for crop production in many areas of the world (Zhu, 2000; Munns, 2002). Salinity involves an increase in the concentration of dissolved salts in the soil water, causing an osmotic effect which may restrict water uptake by plants (Walker, 1994). Furthermore, a negative relationship exists between depression of leaf water potential and the salt concentration in the irrigation solution (Downton and Loveys, 1981). Therefore, salinity impedes with plant transpiration and photosynthesis due to a decrease in the osmotic potential of the soil solution (Munns and Termaat, 1986; Shannon and Grieve, 1999). Walker *et al.,* (1981) reported stomatal closure, induced by salinity, to result in a reduction in photosynthesis and shoot growth. Factors that determine the degree to which salt injury occurs include: salt concentration and ion composition of the saline solution, as well as the period of time that plans are exposed to the saline conditions (Munns, 2002). Saline conditions can cause restrictions in plant growth or even plant death, depending on the concentration (Greenway and Munns, 1980; Munns, 2002; Volkmar *et al.,* 1998).

The foregoing indicates that if wastewater irrigation causes saline soil conditions and a subsequent decrease in plant water potential, it may lead to reduced plant vegetative growth or even plant death. Furthermore, reduced vegetative growth may impact on various aspects of crop production and product quality.

### 2.4.3 Effect on plant nutrient status

Wastewater contains higher amounts of certain nutrients than fresh irrigation water (Neilsen *et al*., 1989a; Lapeña *et al*., 1995; Mulidzi *et al.,* 2009a, b). Wastewater composition however varies between different locations and between different sources of wastewater. Many of the nutrients that are found in wastewater are vital for plant growth. Irrigation with nutrient rich

wastewater may therefore cause an increased plant nutrient status (Laurenson *et al.,* 2010; Stevens *et al.,* 2011). The plant nutrients that are affected by wastewater irrigation are: N, P, K, Ca, Mg, Mn, Na, Cl and B (Neilsen *et al*., 1989a, 1991; Lapeña *et al*., 1995; Laurenson *et al.,* 2010).

Most types of wastewater have higher organic matter and N contents than raw irrigation water (Neilsen *et al*., 1989a, 1991; Lapeña *et al*., 1995; Malandra *et al*., 2003). The higher organic matter content serves as additional nitrogen (N) source for plants which caused elevated plant N levels in *Citrus* trees and sweet cherries irrigated with municipal wastewater (Neilsen *et al*., 1991; Lapeña *et al*., 1995). Wastewater may contribute to the accumulation of organic matter up to 59% (Pedrero & Alarcón, 2009). On the contrary, Neilsen *et al.* (1989b) reported no difference in plant N levels in Riesling grapes when irrigating with municipal wastewater compared to raw water. If irrigation with wastewater increases plant N levels it will lead to increased vegetative growth (Dawoud, 2006). As the foregoing suggests, increases in plant N due to wastewater irrigation is too variable and small for wastewater irrigation to serve as a major source of N nutrition for crops or to replace nitrogen fertilization. In addition, winery wastewater does not usually contain high concentrations of N.

Municipal wastewater often contains higher amounts of phosphorus (P) than raw water (Neilsen *et al*., 1989a, b). The higher P levels may cause elevated P levels in plants irrigated with wastewater. Phosphorus is one of the major macronutrients required by plants for growth and production. Thus, increasing P in plants may lead to enhanced plant growth and reproduction. Furthermore, high plant P concentrations may lead to reduced nodulation in legumes as well as Zn and Cu deficiencies and interference with sugar metabolism (Rossiter, 1955; Silber et al., 2002). Municipal wastewater irrigation was found to enhance leaf P levels in Riesling grapes (Neilsen *et al*., 1989b). In line with these findings a 6% increase in leaf P was observed when sweet cherries were irrigated with municipal wastewater (Neilsen *et al*., 1991). Irrigation with wastewater can serve as an additional P source for crops, promoting plant growth and reproduction.

Wastewater, especially winery wastewater, usually contains elevated potassium (K) levels when compared to common water used for crop irrigation (Lapeña *et al*., 1995; Mulidzi *et al.,* 2009a, b; Sheridan *et al.,* 2011). Due to its importance as co-factor and in maintaining osmotic relations, K is an important nutrient for optimal plant growth, root development and its ability to fight disease (Mulidzi *et al.,* 2009b). However, excessive K consumption can cause cation imbalances which in turn lead to reduced fruit quality in deciduous fruit as well as grape vinification (Mulidzi *et al.,* 2009b). Specifically, excessive K consumption by grapevines results in increased K levels in plant tissue as well as increased juice and wine pH (Mattick *et al.,* 1972;

Morris *et al*., 1983; Laurenson *et al.,* 2010). Municipal wastewater irrigated Riesling grapes, *Citrus* trees, sweet cherries and apple trees had higher leaf K levels than those irrigated with raw water (Neilsen *et al*., 1989a, b, 1991; Lapeña *et al*., 1995). Irrigation using K rich wastewater has been found to increase grapevine petiole K as well (McCarthy, 1981; Neilsen *et al.,* 1989b). These findings indicate that wastewater irrigation can serve as an additional K source for crops and may possibly increase plant health, if insufficient levels are present in the soil. Potassium deficiencies may lead to reduced photosynthetic rate, due to low chlorophyll content, poor chlorophyll ultrastructure and restricted saccharide translocation (Zhao *et al.,* 2001). In addition, high levels of K may also increase juice and wine pH.

The secondary macronutrients that are affected by wastewater irrigation are calcium (Ca) and magnesium (Mg). The leaf Ca levels of Riesling grapes and apple trees are increased by municipal wastewater irrigation (Neilsen *et al*., 1989a, b). However, when C*itrus* trees were irrigated with municipal wastewater no increase in leaf Ca was found (Lapeña *et al*., 1995). Municipal wastewater irrigation decreased leaf Mg content in Riesling grapes, sweet cherry and apple trees (Neilsen *et al*., 1989a, b, 1991). Wastewater had no effect on leaf Mg content in *Citrus* trees (Lapeña *et al*., 1995). Mg was however still present above its required range. Therefore, wastewater irrigation did not have a negative impact on plant health by causing Mg shortages. As neither of these nutrients are altered to beyond their normal ranges, the small increase in leaf Ca and decrease in leaf Mg due to wastewater irrigation does not have a significant impact on crop production.

The micronutrients that are affected by wastewater irrigation are boron (B), chloride (Cl), sodium (Na) and manganese (Mn). Municipal wastewater irrigation increased leaf B content of C*itrus* trees and sweet cherries (Neilsen *et al*., 1991; Lapeña *et al*., 1995). The B content did not however exceed critical toxic levels for these crops. The Cl and Na content in the leaves of C*itrus* trees increased due to municipal wastewater irrigation, but did not exceed its critical toxic level (Lapeña *et al*., 1995). When winery wastewater irrigation was applied to barley, phalaris and lucerne it increased root sodium levels (Mosse *et al*., 2010). Irrigation using Na rich water also causes an increase in plant tissue and juice Na levels, thus resulting in increased juice pH in grapes (Somers, 1975; Stevens *et al.,* 2011). Although wastewater irrigation causes an increase in plant B, Cl and Mn, they are still present within their recommended concentrations. It is important to monitor the micronutrient status of the plant to prevent phytotoxicity, especially B toxicity and excessive Na concentrations, from occurring. High levels of B have been proven to reduce tree growth and productivity while contributing to defoliation and the yellowing of leaves (Aucejo *et al*., 1997). Irrigation with wastewater does not seem to have a significant effect on any other plant nutrients.

### 2.4.4 Effect on vegetative growth

Plant growth rate is a very important factor in numerous criteria of crop production. It is influenced by a number of factors including plant water status, nutrient status and nutrient availability (Aminifard *et al.,* 2010). These factors can be influenced by wastewater irrigation through increasing or decreasing the availability of certain nutrients for plant growth as discussed earlier. Municipal wastewater irrigation was found to promote growth of cherry trees for the first two years of application and was found to have no effect on tree growth after four years of application (Neilsen *et al*., 1991). Irrigation with untreated, undiluted and diluted winery wastewater was found to inhibit vegetative growth of barley, millet, lucerne and phalaris (Mosse *et al*., 2010). Furthermore, biomass production steadily decreased as winery wastewater concentrations increased. A fourfold decrease in vegetative growth was found from 0% to 100% wastewater application. The decrease in vegetative growth was related to a phytotoxic effect. In addition, winery wastewater contains high Na concentrations which may cause a decrease in the osmotic potential of the soil solution, interfering with plant water uptake and resulting in decreased tempos of transpiration, photosynthesis and a decrease in plant growth and productivity (Walker *et al.,* 1981; Munns and Termaat, 1986; Shannon and Grieve, 1999; Kumar *et al.,* 2009) This suggests that excessive amounts of nutrients were applied through winery wastewater irrigation and that one should consider the wastewater composition before application onto crops to avoid phytotoxic effects. The increased vegetative growth of crops due to wastewater irrigation may be related to increased P and K nutrition as both are essential macronutrients for plants. The increased nutrient supply from wastewater which caused increased vegetative growth in some cases is an indication that beneficial wastewater application is possible.

### 2.4.5 Effect on yield

The effect of wastewater on soil and plant nutrient status may influence crop production in terms of yield. Some of the nutrients that are increased by wastewater irrigation may improve vegetative growth and crop health which can lead to increased fruit or crop yield. On the contrary, excessive vegetative growth may result in decreased yield in certain crops. Excessive vegetative growth in grapevines may lead to a decrease in yield as photosynthetic products are translocated to actively growing shoot tips at the expense of bunches (Winkler, 1974). Furthermore, increased shading due to excessive vegetative growth may lead to decreased grapevine yield and reduced cluster size (Smart *et al.,* 1990; Cartechini and Palliotti, 1995). If some of these nutrients are increased excessively it may lead to phytotoxicity and a resulting decrease in yield. Municipal wastewater irrigation significantly increased the cluster size and yield of Riesling grapes in two out of three years (Neilsen *et al*., 1989). The yield increase may have been caused by increased P and K levels resulting from wastewater irrigation. Municipal wastewater irrigation increased apple tree trunk diameter and tree size (Neilsen *et al*., 1989).

The increased tree size was paralleled by a significant increase in yield and number of fruit (Neilsen *et al*., 1989). The increased yield of apple trees may have been caused by increased N, P and K nutrition from wastewater (Osman & AboHassan, 2010). Wastewater irrigation doubled eggplant yield when compared to fresh water irrigation (Al-Nakshabandi *et al.,* 1997). When gobhi sarson was irrigated with distillery wastewater, at different concentrations, yield increased from raw water to 20% wastewater and then decreased with a further increase in wastewater concentration. Minimum yield was obtained at 100% wastewater irrigation (Malaviya & Sharma, 2010). This may indicate that optimum nutrient availability was found at 20% wastewater irrigation after which excessive nutrients cause phytotoxicity and a decrease in yield. Similar results were found by Singh *et al.* (2002) with pulp and paper mill wastewater irrigation on wheat. The contradictory results are probably due to differences in wastewater composition and plant genera. If the wastewater composition is of such nature that nutrients are increased, but not to such an extent that phytotoxicity occurs, wastewater irrigation may increase yield.

### 2.4.6 Effect on fruit quality and juice composition

Wastewater irrigation has a noteworthy effect on crop vegetative growth and nutrient composition. Therefore it could be assumed that wastewater irrigation should affect fruit composition and quality as well. If wastewater irrigation increase vegetative growth to such an extent that excessive shading occurs, it may delay ripening and cause a reduction in berry quality of grapevines (Smart *et al.,* 1990; Cartechini and Palliotti, 1995). Berry colour and total soluble solids (TSS) may be expected to decrease, while total titratable acidity (TTA) and pH may be increased in canopies receiving more shade (Cartechini and Palliotti, 1995). When crops are irrigated using Na and/or K rich water such as wastewater, it may lead to increased concentrations of these elements in plant tissue (McCarthy, 1981; Neilsen *et al.,* 1989; Stevens *et al.,* 2011). When these increases occur in grapevines it causes a decrease in berry tartaric acid content. Thus, juice and wine with high pH values are produced (Somers, 1975; Mpelasoka *et al.,* 2003; Stevens *et al.,* 2011). Increased pH is generally considered negative to juice and wine quality resulting in flat wines and red wines with an undesirable brownish hue (Gladstones, 1992; Rühl, 1989; Mpelasoka *et al*., 2003). In a study conducted by Neilsen *et al.* (1989) municipal wastewater irrigation significantly increased juice pH and soluble solids of Riesling grapes. In contrary to these results Al-Lahham *et al.* (2003) found that municipal wastewater irrigation had no effect on the pH of tomato and soluble solids were decreased. Wastewater irrigation on tomatoes also caused a decline in fruit firmness and an increase in fruit size and weight (Al-Lahham *et al.,* 2003). In accordance with these findings 'Delicious' apples irrigated with municipal wastewater showed increased fruit size resulting in higher quality fruit (Neilsen *et al*., 1989).

All over fruit quality may be affected by wastewater irrigation resulting in increased quality in terms of fruit size or decreased quality due to higher fruit pH. The specific effects on fruit quality are highly dependent on wastewater composition and the crop being irrigated. The foregoing suggests that wastewater can be used for beneficial crop irrigation to enhance the quality of certain fruit crops if applied judiciously.

## 2.5 LEGAL REQUIREMENTS FOR THE USE OF WINERY WASTEWATER IN AGRICULTURE

There are currently three main methods available for disposal of winery wastewater as stated in the National Water Act, 1998. The first method is the discharge to land which includes irrigation with wastewater, as well as evaporation of wastewater by means of evaporation ponds. The second and third methods of disposal are discharge to a water resource and discharge to a sewer, respectively. If a winery wants to implement any of the above mentioned methods of disposal, the disposal method must be authorised by the DWA as per the requirements of the National Water Act, 1998 (Department of Water Affairs and Forestry, 2004; Van Schoor, 2001b; Winetech, 2003).

Strict government legislation according to section 39 of the National Water Act (1988) ensures that untreated winery wastewater will rarely if ever qualify for discharge into a natural water resource. Therefore, wastewater must be treated prior to disposal into a natural water resource. Alternatively, another means of disposal must be performed. According to Van Schoor (2004), more than 95% of existing wineries in South Africa irrigate their winery wastewater onto land by means of sprinkler irrigation systems. The wastewater is mainly irrigated onto kikuyu grass pastures. However, if wineries implement environment friendly procedures it is often possible to irrigate gardens, shrubs, trees and even grapevines beneficially after limited treatment of wastewater (Van Schoor, 2005). In contrast, even the high tolerance kikuyu grass may suffer damage if is over irrigated or irrigated with high strength wastewater.

When irrigation with winery wastewater is being considered there are two primary factors to consider: wastewater composition and irrigation volume. Both of these factors must comply with above mentioned legislation. Furthermore, they are inversely proportional to one another. When the volume of water being irrigated is increased, the irrigation water must be of higher quality. The General Authorisation as stated in the Revision of General Authorisations in terms of Section 39 of the National Water Act, 1998 (Act no. 36 of 1998) on 18 March 2004 is as follows (Department of Water Affairs and Forestry, 2004):

“A person who-

(a) owns or lawfully occupies property registered in the Deeds Office as at the date of this notice;

(b) lawfully occupies or uses land that is not registered or surveyed; or

(c) lawfully has access to land on which the use of water takes place,

may on that property or land

(i) irrigate up to 2000 cubic metres of domestic and biodegradable industrial waste water on any given day provided the-

(a) faecal coliforms do not exceed 1000 per 100 ml;

(b) Chemical Oxygen Demand (COD) does not exceed 75 mg/l;

(c) pH is not less than 5,5 or more than 9,5 pH units;

(d) Ammonia (ionised and un-ionised) as Nitrogen does not exceed 3 mg/l;

(e) Nitrate/Nitrite as Nitrogen does not exceed 15 mg/l;

(f) Chlorine as Free Chlorine does not exceed 0,25 mg/l;

(g) Suspended Solids does not exceed 25 mg/l;

(h) Electrical Conductivity does not exceed 70 milliSiemens (mS) above intake to a maximum of 150 milliSiemens per metre (mS/m);

(i) Ortho-Phosphate as phosphorous does not exceed 10 mg/l;

(j) Fluoride does not exceed 1 mg/l; and

(k) Soap, oil or grease does not exceed 2,5 mg/l.

(ii) irrigate up to 500 cubic metres of domestic or biodegradable industrial wastewater on any given day, provided the-

(a) electrical conductivity does not exceed 200 mS/m;

(b) pH is not less than 6 or more than 9 pH units;

(c) Chemical Oxygen Demand (COD) does not exceed 400 mg/l after removal of algae;

(d) faecal coliforms do not exceed 100 000 per 100 ml; and

(e) Sodium Adsorption Ratio (SAR) does not exceed 5 for biodegradable industrial wastewater;

(iii) irrigate up to 50 cubic metres of biodegradable industrial wastewater on any given day, provided the-

(a) electrical conductivity does not exceed 200 mS/m;

(b) pH is not less than 6 or more than 9 pH units;

(c) Chemical Oxygen Demand (COD) does not exceed 5 000 mg/l after removal of algae;

(d) faecal coliforms do not exceed 100 000 per 100 ml; and

(e) Sodium Adsorption Ratio (SAR) does not exceed 5 for biodegradable industrial wastewater,

if the irrigation of wastewater-

(aA) does not impact on a water resource or any other person's water use, property or land; and

(aB) is not detrimental to the health and safety of the public in the vicinity

of the activity."

(Van Schoor, 2005) "In all the above instances:

- Irrigation may only take place above the 100 year flood line or at a distance greater than 100 meters from the edge of a water resource or borehole which is used for drinking water or stock watering, whichever is the greatest.
- No contamination of ground- or surface water may take place.
- The winery must measure the quantity of wastewater irrigated on a weekly basis.
- The winery must measure the quality of the irrigated wastewater on a monthly basis. Samples should be drawn from the irrigation system from a point located immediately prior to the emitters.
- Written records concerning irrigated wastewater quantities and qualities must be kept for inspection by the responsible authority (DWAF or IPW Auditor) or sent to them on request.
- Existing, as well as possible or proposed irrigation areas must be demarcated on a 1: 10 000 orthophoto and a 1: 50 000 topographic map. Details of the crops under irrigation, irrigation techniques and details of emergency procedures must also be recorded.
- Waterlogging, damage to soil, the occurrence of flies and mosquitoes, bad odours, secondary pollution, penetration of any surface resources and unauthorised use of water by members of the public must be prevented at all times.
- Solid particles must be removed before irrigation and disposed of safely and efficiently.
- Stormwater (rain water) originating from the irrigation area must be collected to prevent contamination of any surface water resource" (Van Schoor, 2005).

"If more than 1000 $m^3$ wastewater is to be stored for subsequent disposal by beneficial irrigation on any given day (up to a maximum of 10 000 $m^3$ per property or up to 50 000 $m^3$ per wastewater dam system) the winery must register for this water use. If more than 500 $m^3$ is to be stored on any given day for recycling purposes, it must also be registered (a maximum of 5 000 $m^3$ will be allowed). Registration is also mandatory where more than 50 $m^3$ wastewater is disposed of in an evaporation pan or wastewater dam system on any given day (a maximum of 1 000 $m^3$ per day will be allowed). The wastewater dams and disposal terrains both have to be situated away from a water source, above the 100-year flood line or alternatively further than 100 meters from the edge of a water resource or borehole used for drinking water or stock

watering, whichever is the greater distance. This authorisation is valid for five years from the date of publication unless the date of authorisation is extended. If any storage dam exceeds a capacity of 50 000 $m^3$, and if the wall of the dam has a vertical height of more than 5 m, it is declared as a dam with an associated safety risk. Such a dam must be registered as such in terms of Sections 117 and 120 of the National Water Act, 1998".

The following guidelines for vineyard irrigation (Table 2.4) with winery wastewater were developed by Ryder (1995). Although these norms represent guidelines in terms of water quality there are many other contributing factors to take into account before vineyard irrigation is performed. These factors include irrigation quantity and frequency, climatic conditions and soil properties.

The majority (>95%) of South African wine cellars dispose of wastewater by means of irrigation (Van Schoor, 2005). When taking into account the wastewater composition of the average South African winery it is apparent that most wineries are unable to perform beneficial crop irrigation with their untreated winery wastewater (Table 2.2). Therefore, several treatment strategies have been developed to enhance the water quality of winery wastewater. Basic treatment options to improve wastewater quality include the addition of chlorine-free hydrated lime to increase wastewater pH and/or the installation of mechanical aerators for reducing wastewater COD (Van Schoor, 2005). More technologically advanced treatment options include biological aerobic systems, aeration of wastewater dams, upflow anaerobic sludge blanket (UASB), biological anaerobiosis, sequence batch reactors, combined aerobic/anaerobic systems, artificial wetlands (reed beds), sequencing batch biofilm reactors, activated sludge, bacterial beds, biological disks, physio-chemical treatment, membrane techniques and reverse osmosis, evapo-concentration to fractional condensation (ECCF) and a combination of these processes (Andreottola *et al.,* 2002; Petruccioli *et al.,* 2002; Van Schoor, 2005; Kumar *et al.*, 2009). Each one of these treatment systems has a different purpose. In order to improve the targeted water quality parameter the correct treatment system needs to be installed. However, the treatment of winery wastewater is not necessarily a sustainable option as it is expensive and associated with high energy use and the emission of greenhouse gasses which may have a major impact on the carbon footprint in wine-producing areas (Rosso *et al.,* 2009).

**Table 2.4** Reclaimed effluent quality standards for vineyard re-use (Ryder, 1995).

| Parameter | Units | Optimum value | Maximum values |
|---|---|---|---|
| pH | (KCl) | 6.5 - 8.4 | 6.0 – 9.0 |
| EC | mS/m | < 75 | < 150 |
| Total dissolved solids | mg/L $CaCO_3$ | < 500 | < 1000 |
| Alkalinity | mg/L $CaCO_3$ | < 150 | < 250 |
| Hardness | mg/L | < 250 | < 400 |
| Ca | mg/L | < 60 | < 100 |
| Mg | mg/L | < 25 | < 50 |
| Na | mg/L | < 65 | < 100 |
| K | mg/L | < 5 | < 10 |
| Fe | mg/L | < 5 | < 5 |
| Mn | mg/L | < 0.2 | < 0.5 |
| Cu | mg/L | < 0.01 | < 0.05 |
| Zn | mg/L | < 2 | < 5 |
| Bicarbonate | mg/L | < 200 | < 300 |
| Carbonate | mg/L | < 5 | < 10 |
| Chloride | mg/L | < 70 | < 120 |
| Sulphate | mg/L | < 150 | < 250 |
| N | mg/L | < 5 | < 10 |
| P | mg/L | < 5 | < 10 |
| B | mg/L | < 0.5 | < 1 |
| SAR | | < 6 | < 9 |
| COD* | mg/L | < 60 | < 100 |
| Coliforms | MPN/100ml | < 23 | < 230 |

* Adjusted from biological oxygen demand (BOD) where BOD = 66% of COD.

The most important parameters used for determining wastewater quality and their allowed ranges for irrigation are displayed in Table 2.5.

**Table 2.5** Legal requirements for pH, sodium adsorption ratio, chemical oxygen demand and electrical conductivity in irrigation water (Department of Water Affairs and Forestry, 2004).

| Parameter and unit | Legal requirements for irrigation |
|---|---|
| pH | Between 6 & 9 |
| SAR | <5 |
| COD (mg/L) | <5000, 400 or 75* |
| EC (mS/m) | <200 |

* Varies according to volume irrigated on any given day

## 2.6 CONCLUDING REMARKS

As pressure on existing water resources mount, alternative sources for vineyard irrigation need investigation. South African wine cellars struggle to produce winery wastewater of acceptable quality for beneficial vineyard irrigation to commence. Therefore, they irrigate to dispose of wastewater, rather than applying beneficial crop irrigation practices. Irrigation using low quality undiluted and even diluted wastewater may have negative implications for crop production while also having detrimental impacts on the soil. Furthermore, treatment of winery wastewater is expensive and harmful to the environment and therefore may not be a sustainable option. For these reasons, further research in the field of winery wastewater irrigation and the possibility of augmenting winery wastewater for irrigation purposes is required. The augmentation of wastewater prior to irrigation may become compulsory practise in the near future if it can be established as an effective means of wastewater disposal. Furthermore, if irrigation with these enriched water supplies can reduce fertilization costs it will help in reducing wine production costs.

## 2.7 LITERATURE CITED


Al-Lahham, O., El Assi, N. & Fayyad, M., 2003. Impact of treated wastewater irrigation on quality attributes and contamination of tomato fruit. Agr. Water Manage. 61, 51-62.

Al-Nakshabandi, G., Saqqar, M., Shatanawi, M., Fayyad, M. & Al-Horani, H., 1997. Some environmental problems associated with the use of treated wastewater for irrigation in jordan. Agr. Water Manage. 34, 81-94.

Aminifard, M.H., Aroiee, H., Fatemi, H., Ameri, A. & Karimpour, S., 2010. Responses of eggplant (*Solanum melongena* L.) to different rates of nitrogen under field conditions. J. Cent. Eur. Agric. 11, 453-458.

Andreottola, G., Foladori, P., Raqazzi, M. & Villa, R., 2002. Treatment of winery wastewater in a sequencing batch biofilm reactor. Water Sci. Technol. 45, 347-354.

Aucejo, A., Ferrer, J., Gabaldón, C., Marzal, P. & Seco, A., 1997. Diagnosis of boron, fluorine, lead, nickel and zinc toxicity in citrus plantations in Villarreal, Spain. Water Air Soil Poll. 94, 349-360.

Caballero, A.R., 2010. Study of bacterial communities – a wastewater treatment perspective. Thesis, School of Sustainable Development of Society and Technology, Mälardalen University, Västerås, Sweden.

Cartechini, A. & Palliotti, A., 1995. Effect of shading on vine morphology and productivity and leaf gas exchange characteristics in grapevines in the field. Am. J. Enol. Vitic. 46, 227-234.

Chapman, J.A., 1996. Cleaner production for the wine industry. South Australian Wine and Brandy Industry Association, Adelaide, Australia.

Chapman, J.A., Baker, P. & Wills, S., 2001. Winery wastewater handbook: production, impacts, and management. Winetitles, Adelaide.

Coetzee, G., Malandra, L., Wolfaardt, G.M. & Viljoen-Bloom, M., 2004. Dynamics of a microbial biofilm in a rotating biological contactor for the treatment of winery effluent. Water SA 30, 407-412.

Dawoud, D.H., Raouf, F.A. & Ali salih, A., 2006. Effect of different rates of nitrogen fertilizer on growth, fruit quality and yield of guava under New Halfa conditions. Shamabat Research Station, Agricultural Research Corporation, Khartoum North, Sudan. pp. 209-214. (http://www.arcsudan.sd/).

Department of Environmental Affairs and Tourism, 2004. A national climate change response strategy for South Africa. *(*unfccc.int/files/meetings/seminar/.../pdf/sem_sup3_south_africa.pdf).

Department of Water Affairs and Forestry, 2004. Revision of general authorisation in terms of Section 39 of the National Water Act, 1998 (Act 36 of 1998). Section 21, Government Notice 1091, Government Gazette 26187, 13, 1-33. March, 2004. Department of Water Affairs and Forestry, Pretoria, South Africa.

Downton, W.J.S. & Loveys, B.R., 1981. Abscisic acid content and osmotic relations of salt-stressed grapevine leaves. Aust. J. Plant Physiol. 8, 443-452.

Eusébio, A., Mateus, M., Baeta-Hall, L., Almeida-Vara, E. & Duarte, J.C., 2005. Microflora evaluation of two agro-industrial effluents treated by the JACTO jet-loop type reactor system. Water Sci. Technol. 51, 107-112.

Gladstones, J., 1992. Viticulture and environment: A study of the effects of environment on grape-growing and wine qualities, with emphasis on present and future areas for growing winegrapes in Australia. Winetitles, Adelaide.

Glaetzer, S.J., 2000. Environmental management case study: Winery. Proc. 10th Aust. Wine Ind. Tech. Conf., Adelaide, Australia.

Greenway, H. & Munns, R., 1980. Mechanisms of salt tolerance in non halophytes. Ann. Rev. Plant Physiol. 31, 149-190.

Jourjon, F., Racault, Y. & Rochard, J., 2001. Effluents vinicoles: gestion et traitements. Editions Feret, Bordeaux.

Jourjon, F., Khaldi, S., Reveillere, M., Thibault, C., Poulard, A., Chretien, P. & Bednar, J., 2005. Microbiological characterization of winery effluent: an inventory of the sites for different treatment systems. Water Sci. Technol. 51, 19-26.

Kumar, A., Arienzo, M., Quayle, W., Christen, E., Grocke, S., Fattore, A., Doan, H., Gonzago, D., Zandonna, R., Bartrop, K., Smith, L., Correl, R. & Kookana, R., 2009. Developing a systematic approach to winery wastewater management. CSIRO Land and Water Science Report. pp. 1-131.

Lapeña, L., Cerezo, M. & García-Augustin, P., 1995. Possible reuse of treated municipal wastewater for citrus spp. plant irrigation. Bull. Environ. Contam. Toxicol. 55, 697-703.

Laurenson, S., Bolan, N., Smith, E. & McCarthy, M., 2010. Winery wastewater irrigation: effects of sodium and potassium on soil structure. CRC CARE Technical Report 19, 1-25. (http://www.crccare.com/publications/downloads/CRC-CARE-Tech-Report-19.pdf).

Liu, R., Zhou, Q., Zhang, L. & Guo, H., 2006. Toxic effects of monosodium glutamate wastewater on crop seed germination and root elongation. Ying Yong Sheng Tai Xue Bao 17, 1286-1290.

Malandra, L., Wolfaardt, G., Zietsman, A., & Viljoen-Bloom, M., 2003. Microbiology of a biological contactor for winery wastewater treatment. Water res. 37, 4125-4134.

Malaviya, P. & Sharma, A., 2010. Effect of distillery effluent on yield attributes of brassica napus L. J. Environ. Biol. 32, 380-385.

Mattick, L.R., Shaulis, N.J. & Moyer, J.C., 1972. The effect of potassium fertilization on the acid content of 'Concord' grape juice. Am. J. Enol. Vitic. 23, 26-30.

McCarthy, M.G., 1981. Irrigation of grapevines with sewage effluent. I. Effects on yield and petiole composition. Am. J. Enol. Vitic. 32, 189-196.

Moncault, E., 2003. Communications personnelles sur le suivi de station d'épuration de la ville de Paris. As cited by: Jourjon, F., Khaldi, S., Reveillere, M., Thibault, C., Poulard, A., Chretien, P. & Bednar, J.,

2005. Microbiological characterization of winery effluent: an inventory of the sites for different treatment systems. Water Sci. Technol. 51, 19-26.

Morris, J., Sims, C. & Cawthon, D., 1983. Effects of excessive potassium levels on pH, acidity and color of fresh and stored grape juice. Am. J. Enol. Vitic. 34, 35-39.

Mosse, K.P.M., Patti, A.F., Christen, E.W. & Cavagnaro, T.R., 2010. Winery wastewater inhibits seed germination and vegetative growth of common crop species. J. Hazard. Mater. 180, 63-70.

Mpelasoka, B.S., Schachtman, D.R., Treeby, M.T. & Thomas, M.R., 2003. A review of potassium nutrition in grapevines with special emphasis on berry accumulation. Aust. J. Grape Wine Res. 9, 154-168.

Mulidzi, A.R., Wooldridge, J., Laker, M.C. & Van Schoor, L., 2009a. Composition of effluents from wineries in the Western and Northern Cape Provinces I. Seasonal variations and differences between wineries. Wineland, January. pp. 88-91.

Mulidzi, A.R., Wooldridge, J., Laker, M.C. & Van Schoor, L., 2009b. Composition of effluents from wineries in the Western and Northern Cape Provinces II. Impacts on soil and environment. Wineland, February. pp. 61-67.

Munns, R., 2002. Comparative physiology of salt and water stress. Plant Cell Environ. 25, 239-250.

Munns, R. & Termaat, A., 1986. Whole-plant responses to salinity. Aust. J. Plant Physiol. 13, 143-160.

Neilsen, G., Stevenson, D., Fitzpatrick, J. & Brownlee, C., 1989a. Nutrition and yield of young apple trees irrigated with municipal waste water. J. Am. Soc. Hortic. Sci. 114, 377-383.

Neilsen, G., Stevenson, D. & Fitzpatrick, J., 1989b. The effect of municipal wastewater irrigation and rate of N fertilization on petiole composition, yield and quality of Okanagan Riesling grapes. Can. J. Plant Sci. 69, 1285-1294.

Neilsen, G., Stevenson, D., Fitzpatrick, J. & Brownlee, C., 1991. Soil and sweet cherry responses to irrigation with wastewater. Can. J. Soil Sci. 71, 31-41.

Osman, H.E. & AboHassan, A.A., 2010. Effect of NPK fertilization on growth and dry matter accumulation in mangrove [Avicennia marina (Forssk) vierh] grown in Western Saudi Arabia. JKAU: Met., Env. & Arid Land Agric. Sci. 21, 57-70.

Pedrero, F. & Alarcón, J.J., 2009. Effects of treated wastewater irrigation on lemon trees. Desalination 246, 631-639.

Petruccioli, M., Duarte, J.C. & Federici, F., 2000. High-rate aerobic treatment of winery wastewater using bioreactors with free and immobilized activated sludge. J. Biosci. Bioeng. 90, 381-386.

Petruccioli, M., Duarte, J.C., Eusébio, A. & Federici, F., 2002. Aerobic treatment of winery wastewater using a jet-loop activated sludge reactor. Process Biochem. 37, 821-829.

Racault, Y. & Lenoir, A., 1994. Evolution des charges polluantes de deux caves vinicoles du Sud-Ouest de la France sur un cycle annuel. In: Cemagref (ed). Congre`s International Effluents vinicoles. Narbonne, France.

Rossiter, R.C., 1955. The influence of soil type on phosphorus toxicity in subterranean clover (Trifolium subterraneum). Aust. J. Agr. Res. 6, 1-8.

Rosso, D. & Bolzonella, D., 2009. Carbon footprint of aerobic biological treatment of winery wastewater. Water Sci. Technol. 60, 1185-1189.

Rühl, E.H., 1989. Effect of potassium and nitrogen supply on the distribution of minerals and organic-acids and the composition of grape juice of Sultana vines. Aust. J. Exp. Agr. 29, 133-137.

Ryder, R.A., 1995. Aerobic pond treatment of winery wastewater for vineyard irrigation by drip and spray system in California. Rev. Fr. Oenol. 152, 22-24.

Shannon, M.C. & Grieve, C.M., 1999. Tolerance of vegetable crops to salinity. Sci. Hortic. 78, 5-38.

Sheridan, C., 2007. Constructed wetlands for the primary treatment of winery effluent. MSc thesis, University of Cape Town, Private Bag X3, 7701 Rondebosch, South Africa.

Sheridan, C.M., Glasser, D., Hildebrandt, D., Petersen, J. & Rohwers, J., 2011. An annual and seasonal characterisation of winery effluent in South Africa. S. Afr. J. Enol. Vitic. 32, 1-8.

Silber, A., Ben-Jaacov, J., Ackerman, A., Bar-Tal, A., Levkovitch, I., Matsevitz-Yosef, T., Swartzberg, D., Riov, J. & Granot, D., 2002. Interrelationship between phosphorus toxicity and sugar metabolism in Verticordia plumose L. Plant Soil 245, 249-260.

Singh, A., Aggarwal, C., Rai, J. & Singh, P., 2002. Assessment of pulp and paper mill effluent on growth, yield and nutrient quality of wheat (Triticum aestivum L.). J. Environ. Biol. 23, 283-288.

Smart, R.E., Dick, J.K., Gravett, M. & Fisher, B.M., 1990. Canopy management to improve grape yield and wine quality – principles and practices. S. Afr. J. Enol. Vitic. 11, 3-17.

Somers, T.C., 1975. In search of quality for red wines. Food Technol. Aust. 27, 49-56.

Stevens, R.M., Harvey, G. & Partington, D.L., 2011. Irrigation of grapevines with saline water at different growth stages: effect on leaf, wood and juice composition. Aust. J. Grape Wine Res. 17, 239-248.

Van Schoor, L.H., 2000. Management options to minimise negative environmental impacts at wine cellars. Wineland, July. pp. 97-100.

Van Schoor, L.H., 2001a. Proposed IPW criteria for managing wastewater, solid waste, noise and air pollution. Wineland, May. pp. 4.

Van Schoor, L.H., 2001b. Environmental legislation in the viticultural and wine industry. Wineland, January. pp. 114-117.

Van Schoor, L.H., 2004. A prototype ISO 14001 environmental management system for wine cellars. MSc thesis, Stellenbosch University, Private Bag X1, 7602 Matieland (Stellenbosch), South Africa.

Van Schoor, L.H., 2005. Guidelines for management of wastewater and solid waste at existing wineries. Winetech, South Africa. (http://www.winetech.co.za/index.php).

Van Schoor, L.H. & Rossouw, J., 2004. Guidelines for a winery wastewater and solid waste management plan. Wineland, March.

Volkmar, K.M., Hu, Y. & Steppuhn, H., 1998. Physiological responses of plants to salinity: a review. Can. J. Plant Sci. 78, 19-27.

Walker, R.R., 1994. Grapevine responses to salinity. Bulletin de l'O. I.V. 67, 634-661.

Walker, R.R., Torokfalvy, E., Steele Scott, N. & Kriedemann, P.E., 1981. An analysis of photosynthetic response to salt treatment in *Vitis vinifera*. Aust. J. Plant Physiol. 8, 359-374.

Wines of South Africa (WOSA), 2011. (http://www.wosa.co.za/sa/stats_worldwide.php).

Winetech, 2003. Integrated environmental management guidelines. Winetech cd: Vol. 4 & 7. Paarl, South Africa.

Winkler, A.J., Cook, J.A., Kliewer, W.M. & Lider, L.A., 1974 ($4^{th}$ ed). General viticulture. University of California Press, Berkeley.

Zhao, D., Oosterhuis, D.M. & Bednarz, C.W., 2001. Influence of potassium deficiency on photosynthesis, chlorophyll content, and chloroplast ultrastructure of cotton plants. Photosynthetica 39, 103-109.

Zhu, J.K., 2000. Genetic analysis of plant salt tolerance using Arabidopsis. Plant Physiol. 124, 941-948.

# Chapter 3

## Research results

### The effect of irrigation using winery wastewater on grapevine growth, berry development and berry composition of Cabernet Sauvignon/99R in the Breede River Valley

# 3. RESEARCH RESULTS

## 3.1 INTRODUCTION

Water availability is one of the most important parameters determining grapevine growth and subsequent wine quality. High irrigation frequencies and the associated high soil water availability cause increases in grapevine vegetative growth when compared to grapevines exposed to water constraints (Van Zyl, 1981; McCarthy *et al.,* 1983; Myburgh, 2011). On the contrary, water shortage is one of the primary factors limiting production in numerous vineyards around the world (Williams *et al.,* 1994; Laurenson *et al.,* 2010). A general shortage of water for irrigation causes a decline in yield and grape quality as a result of soil salinisation as well as a decrease in economic return per unit area of land (Hamilton *et al*., 2005; Anderson *et al.,* 2008). In order to manage grapevine growth and wine quality by effective management of irrigation scheduling, water needs to be readily available for irrigation. Therefore, pressure on available water resources has triggered a greater focus on finding alternative water sources, such as treated winery wastewater, for crop irrigation (Ryder, 1995; Laurenson *et al.,* 2010).

Winery wastewater contains higher concentrations of certain elements than the typical water used for vineyard irrigation (Mulidzi *et al.,* 2009; Sheridan *et al.,* 2011). The dominant metallic species in winery wastewater are sodium (Na), potassium (K), calcium (Ca), magnesium (Mg) and iron (Fe), the most important of these being Na and K (Sheridan *et al.,* 2011). Furthermore, zinc (Zn), copper (Cu), lead (Pb) and manganese (Mn) are present at low concentrations, while chromium (Cr), boron (B) and arsenic (As) are not present at detectable concentrations. The salt concentration of winery wastewater is significantly higher than that of many other water sources used for grapevine irrigation, such as municipal water and river water (Laurenson *et al.,* 2010). The reason for the high Na and K concentrations in winery wastewater when compared to typical sources of irrigation water is primarily due to the use of sodium hydroxide (NaOH) and potassium hydroxide (KOH) cleaners in winery cleaning operations, as well is tartrate crystals washed from tanks after cold stabalisation (Van Schoor, 2005; Kumar *et al.*, 2009).

The high Na and K concentration of winery wastewater may lead to accumulation of these cations in the soils, resulting in soil structure degradation (Mulidzi *et al.,* 2009; Laurenson *et al.*, 2010). Furthermore, Na can displace more desirable cations such as Ca and Mg. The build up of these salts can reduce plant growth and productivity (Kumar *et al.,* 2009). Winery wastewater and saline water are similar in the sense that both contain considerable amounts of Na. Therefore, winery wastewater irrigation can cause saline soil conditions (South Australian Environmental Protection Authority, 2004). When irrigation with saline water is applied it often results in the accumulation of chloride (Cl) and Na to toxic levels for plants (Stevens *et al.,*

2011). This is caused by an osmotic effect where the increase in soluble salt concentration in the soil solution imposes an osmotic drought on the plant, impeding with plant water uptake, transpiration, photosynthesis and growth (Bernstein, 1975; Walker *et al.,* 1981; Marschner, 1986; Munns and Termaat, 1986). The increase of Cl and Na to toxic levels causes ruinous effects on plant growth. Stevens *et al.* (2011) reported that saline water irrigation increases juice Na content, the greatest increase occurring when irrigation was applied from full bloom to véraison. Furthermore, the increase in juice Na is larger when irrigation water containing more salts is applied. The enhanced Na uptake into berries also leads to increased juice pH, affecting wine quality negatively (Somers, 1975; Stevens *et al.,* 2011). In earlier studies drip-irrigated saline water was found to cause a decline in grapevine yield due to decreased grapevine leaf photosynthesis (Prior *et al*., 1992; Stevens *et al*., 1999). A strong negative correlation between leaf photosynthesis and leaf Na and Cl concentration was present. Walker *et al.* (1997) concluded that Cl, rather than Na, was the ion responsible for a decline in photosynthetic activity. Therefore, winery wastewater should not inhibit grapevine photosynthetic activity, as it usually contains acceptable amounts of Cl (Van Schoor and Mulidzi, 2001). However, irrigation with raw and diluted winery wastewater has been found to inhibit vegetative growth of barley, millet, lucerne and phalaris due to a phytotoxic effect (Mosse *et al*., 2010). Calcium and Mg deficiencies in grapevines have also been caused due to increased uptake of Na at the expense of these elements (Grattan and Grieve, 1998).

Although plants generally have high K requirements, the K concentration in winery wastewater far exceeds these requirements (Laurenson *et al.,* 2010). If these high amounts of K are absorbed by the plant it can lead to elevated plant K levels and increased fruit pH (Mattick *et al.,* 1972; Morris *et al*., 1983). It is uncertain to what extent soil K content and berry K content are correlated. Boulton (1980) stated that soil exchangeable K status did not directly affect berry K status. On the contrary, Garcia *et al.* (1999) stated that berry K status is directly affected by soil K status. Despite these varying results, irrigation of K rich wastewater has been found to increase grapevine petiole K (McCarthy, 1981; Neilsen *et al.,* 1989a). Excessive grape K levels leads to the formation of insoluble potassium tartrate salts that cause a decrease in available tartaric acid content. The shortage in berry tartaric acid causes an increase in berry and wine pH (Mpelsoka *et al.,* 2003). Iland and Coombe (1988) also stated that excessive Ca and K fertilization causes a decrease in malic acid concentration, due to salt formation, resulting in a decrease in total titratable acidity (TTA). Another possible effect is that K may be absorbed at the expense of certain other elements. Excessive K application through wastewater irrigation has led to Ca and Mg deficiencies in rye grass pastures (Bolan *et al.,* 2004).

The specific effects of augmented winery wastewater irrigation on grapevine growth, berry development and berry composition have not been studied in detail. If irrigation using

augmented winery wastewater does not affect grapevine growth and berry composition in a negative manner it can help relieve pressure on water resources while providing an effective and environment friendly method of wastewater management. Therefore, the objective of this study was to assess the effect that irrigation using augmented winery wastewater has on grapevine growth, reproductive growth and berry composition of Cabernet Sauvignon in the Breede River Valley. Cabernet Sauvignon is one of the most important red wine cultivars in the South African wine industry.

## 3.2 MATERIALS AND METHODS

### 3.2.1 Experimental vineyard

The field trial was carried out in a *Vitis vinifera* L. cv. Cabernet Sauvignon vineyard grafted onto Richter 99 rootstock in the 2010/11 and 2011/12 seasons. The vineyard is located at Goudini Winery, situated just outside of Rawsonville in the Breede River Valley in the Western Cape. Rawsonville is situated in a class IV climatic region (Winkler *et al*., 1974) at 33°24'47" south latitude and 19˚12'6" east longitude. The grapevines are planted 2.40 m x 1.20 m and are trained onto a five strand, double lengthened, Perold trellis system. The row orientation is north-east to south-west. The grapevines are planted in a sandy alluvial soil. Potassium fertilisation was applied to the soils in both seasons: 30 kg per ha was applied to all treatments in the middle of November in the 2010/11 season, and in the 2011/12 season, 30 kg per ha was applied to treatments 1 to 6 in the middle of December. Potassium fertilisation was applied to avoid K-deficiencies as large amounts of K are leeched from the sandy soil due to winter rains. The vineyard had been irrigated with drip irrigation preceding the trial, but had since been switched to a micro-sprinkler irrigation system. Grapevines were pruned to two bud spurs, spaced *ca.* 12 cm, to ensure that five spurs were present on each cordon arm. A complete suckering action was performed before flowering, after which water shoots were constantly removed from the trunk and cordon arms. The accommodation of shoots between the trellis wires was performed before the end of October. In the beginning of December actively growing shoots were topped at *ca.* 30 cm above the top trellis wire.

### 3.2.2 Experimental layout

Eight different irrigation treatments with winery wastewater, augmented to different levels of COD, were applied and compared to vineyard irrigation with water from the Holsloot River, *ca.* 100 m from the vineyard. In this study, the level of COD in the irrigation water is an indication of water quality, with higher COD levels indicating poorer quality. The target COD concentrations were: 100 mg/L, 250 mg/L, 500 mg/L, 1000 mg/L, 1500 mg/L, 2000 mg/L, 2500 mg/L and 3000 mg/L.

Winery wastewater from the cellar was pumped to a pond near the cellar, after which the COD concentration was determined. The COD concentration of the winery wastewater varied largely between periods of irrigation, ranging from 6000 mg/L to 25000 mg/L. The water from the pond was then filtered and pumped to eight tanks next to the vineyard, each being able to store approximately 13000 L of water. The target COD levels were then obtained by mixing winery wastewater from the pond with raw water according to pre-determined ratios. Depending on the COD of the winery wastewater, a certain percentage of the tank was filled with winery wastewater (6000 mg/L – 25000 mg/L COD) and augmented to its target COD concentration by using raw water (5 mg/L COD). The effluent and the raw water were mixed in the tanks just before each irrigation practice. Approximately one hour into irrigation, water samples for all irrigation treatments were taken and tested to make sure that the COD concentrations of the water corresponded with the target levels. The COD concentrations for all treatments throughout both seasons corresponded with the target COD levels within <2% standard deviation. These water samples were also analysed to determine the chemical and microbial composition of the various irrigation water treatments.

Treatment and repetition layout is displayed in Fig 3.1. Soil fertility is highest in the bottom right corner and decreases towards the top left corner. All vines were initially irrigated with raw water until the winery had produced enough wastewater for the first wastewater irrigation to be applied. Irrigation with winery wastewater was applied from soon after véraison until the first autumn rains started in April. The experimental layout was laid out according to a randomized block design with each treatment being replicated three times. Each experimental plot contained ten experimental grapevines with two buffer grapevines at each end and one buffer row on each side of the plots. Grapevines received the various irrigation treatments approximately once every 14 days, or according to vineyard water requirements. The neutron scattering technique was used to measure soil water content to a depth of 150 cm in increments of 30 cm. A neutron probe (Hydroprobe 503DR, CPN®, California) was used for this purpose. All the vineyards were irrigated simultaneously and received identical volumes of water during irrigations, approximately 41 mm.

| | | | | |
|---|---|---|---|---|
| R1T10 | R1T4 | R2T1 | R2T2 | R3T8 |
| R1T8 | R1T5 | R2T6 | R2T3 | R3T9 |
| R1T9 | R1T3 | R2T10 | R3T1 | R3T5 |
| R1T6 | R1T7 | R2T9 | R3T7 | R3T4 |
| R1T2 | R2T5 | R2T4 | R3T3 | R3T2 |
| R1T1 | R2T8 | R2T7 | R3T6 | R3T10 |

| Treatment no. | Target COD (mg/L) |
|---|---|
| T1 | 5 |
| T2 | 100 |
| T3 | 250 |
| T4 | 500 |
| T5 | 1000 |
| T6 | 1500 |
| T7 | 2000 |
| T8 | 2500 |
| T9 | 3000 |
| T10 | N/a* |

**Fig 3.1** Experimental layout and irrigation water treatments (R = Replication; T = Treatment; COD = Target chemical oxygen demand level).
*N/a – Not applicable

### 3.2.3 Chemical composition of irrigation water

Water quality for all irrigation treatments was determined at each irrigation event throughout the year by a commercial laboratory (Bemlab, Strand), according to methods described by Clesceri *et al.* (1988). The amount of elements, ions and heavy metals contained by the various irrigation treatments was then added together and converted to kg/ha to illustrate the amount applied *via* each irrigation treatment. Average values for EC and SAR were also calculated for each irrigation treatment.

### 3.2.4 Microbial composition of irrigation water

In the 2011/12 season, water used to irrigate the various treatments was plated out on yeast extract agar according to methods described in Chapter 4. Water from each of four periods of irrigation from February up to harvest was plated out. The winery wastewater, as measured before augmentation, contained 25000, 15000, 6000 and 7000 mg/L COD for the four periods of irrigation, respectively. Total yeast and bacterial counts for raw river water, winery wastewater and each of the wastewater irrigation treatments were determined to assess whether the various wastewater irrigation treatments contained larger yeast and bacterial populations than raw water. Furthermore, the possibility that these microbes were transferred into juice and wine, affecting its composition and quality, was investigated as described in Chapter 4.

### 3.2.5 Vegetative growth

Vegetative growth was quantified during two phenological stages of the grapevine. Primary and secondary shoot length and leaf area as well as number of leaves on primary shoots were quantified one day prior to harvest and cane mass was quantified at pruning.

For quantification of primary and secondary shoot length and leaf area, four shoots were randomly sampled from each experimental plot. Shoots were sampled from identically positioned grapevines within an experimental plot and from fixed positions on a grapevine. After sampling shoots were stored in plastic bags and refrigerated to maintain shoot and leaf integrity. Shoots were separated into primary and secondary shoots and shoot lengths measured after removal of leaves. Leaves were grouped into primary and secondary leaves, after which the respective leaf areas were determined using an electronic leaf area meter (LI-COR LI-3100, Lincoln, Nebraska, USA). Leaf area per shoot was quantified by passing all leaves representing one shoot through the leaf area meter and adding their respective areas. Cane mass at pruning was weighed using a hanging balance and converted from kg shoots per experimental plot to tons per hectare.

### 3.2.6 Berry development and composition

3.2.6.1 Berry sampling

Berries were sampled once every ten days from middle-late December up to harvest, in the 2010/11 and 2011/12 seasons. One hundred berries were sampled at random from each of four experimental treatments. Irrigation treatments that were sampled are: raw water, 1000 mg/L, 2000 mg/L and 3000 mg/L COD concentration. These treatments were specifically chosen as they were the four treatments that best represented all nine treatments. If treatment effects were observed in the 2010/11 season, samples would have be taken from all treatments in the 2011/12 season. Berries were sampled from each of the three replications of each treatment. Ten berries were sampled from each of the ten grapevines from each experimental plot. Five berries were sampled from the front side and five berries from the back side of the bunch. Bunches from consecutive grapevines were alternatively taken from shaded and sun exposed parts of the grapevine. Berries were sampled into plastic bags to avoid loss of moisture during transportation to the laboratory. At harvest, berries were sampled from each of the nine treatments according to the same procedure. Once berries arrived at the laboratory they were used to determine berry fresh mass, berry volume, total soluble solids (TSS), TTA and pH.

3.2.6.2 Berry mass and berry volume

Berries were weighed on a digital scale to determine berry fresh mass. Berry volume was determined according to water displacement in a measuring cylinder. Mass per berry and

volume per berry were determined by dividing the total berry mass and berry volume by the number of berries.

3.2.6.3 Juice characteristics

Prior to juice analysis, berries were blotted dry, crushed and centrifuged (Beckman, Model J2-21, Beckman Instruments Inc., Palo Alto, CA, USA) at 10000 rpm for 10 minutes for maximum juice extraction.

3.2.6.3.1 Nitrogen, phosphorus, cation and heavy metal composition

Must ion composition was analysed by a certified commercial laboratory (BEMLAB, Strand) (SANAS accreditation). Methods described by Clesceri *et al.* (1998) were used for determination of nitrogen (N), K, Na, Cl, phosphorus (P), Ca, Mg and heavy metals: Cr, Pb, mercury (Hg), cadmium (Cd) and As content. A nitrogen analyser was used for analysis of N. An ICP-OES spectrometer (PerkinElmer Optima 7300 DV, Waltham, Massachusetts, USA.) was used for analysis of K, Na, Cl, P, Ca, Mg and heavy metals.

3.2.6.3.2 Total soluble solids

Sugar content (°B) was determined by means of refractometry using a digital refractometer (Pocket PAL-1, Atago U.S.A. inc., Bellevue, WA, U.S.A.) (Iland *et al*., 2000).

3.2.6.3.3 pH

For pH determination an automatic titrator (Metrohm 785 DMP Tritino, Metrohm AG, Herisau, Switzerland) with a combined electrode and temperature probe was used. The electrode was calibrated using certified buffers (Crison pH 7.00 and pH 4.00, LASEC, Cape Town, SA). The pH was determined as described by Iland *et al.* (2000).

3.2.6.3.4 Total titratable acidity

Total titratable acidity was measured by means of potentiometric titration using an automatic titrator (Metrohm 785 DMP Tritino, Metrohm AG, Herisau, Switzerland). Samples were titrated to the endpoint pH 7.00 using standardised 0.33 N sodium hydroxide (Merck, Cape Town, SA). The TTA was determined as described by Iland *et al.* (2000) and expressed as g/L tartaric acid.

3.2.6.3.5 Tartaric acid and malic acid

Determination of berry tartaric and malic acids were not included in the initial project proposal and, therefore not determined in the 2010/11 season. Further investigation into irrigation using winery wastewater indicated that organic acid concentrations may be affected by it. Therefore, these organic acids were determined in the 2011/12 season. From 47 days prior to harvest berry malic acid and tartaric acid were quantified. The goal was to take random samples every

10 days. However, delayed berry ripening forced a 36 day interval between the second last sampling and the final sampling at harvest. Five bunches were cut from each experimental plot, each from a different grapevine. Three of these bunches were sun exposed bunches and two were shaded bunches. Bunches were preserved as previously mentioned for berry analysis, after which they were crushed and taken to a commercial laboratory for analysis of malic acid and tartaric acid. Tartaric acid was determined using a tartaric acid enzymatic kit (Boehringer Mannheim, Roche) and expressed as g/L tartaric acid. Malic acid was determined using a malic acid enzymatic kit (Boehringer Mannheim, Roche) and expressed as g/L malic acid. If acids were converted to their respective salt forms, they would not contribute to the titratable acidity.

3.2.6.4 Berry skin characteristics

Berry skin wet mass, dry mass, colour and phenolic composition was determined according to the method described by Hunter *et al.* (1991) with a few adaptations. Two mm quartz cuvettes were used to measure absorbance instead of the 10 mm cuvettes used by Hunter *et al.* (1991). Berry skin colour was measured at 420 nm absorbance ($A_{420}$), in addition to 520 nm absorbance ($A_{520}$), to quantify brown colour pigments.

### 3.2.7 Yield components at harvest

3.2.7.1 Harvesting and reproductive measurements

Grapes were harvested at a target sugar concentration of 24 degree balling (°B). Bunches were hand harvested into plastic crates to avoid damage to grapes. During harvesting the number of bunches harvested from each experimental plot were counted using mechanical counters. Grapes were weighed on a digital scale to determine mean yield per grapevine and values were converted to tonnes per hectare. Bunch mass was determined by dividing total bunch mass per plot by the number of bunches. Berry mass and berry volume were determined as described in Section 3.2.6.2.

### 3.2.8 Statistical analysis

Microsoft® Excel (Microsoft Corporation, USA) was used to sort raw data and to calculate the standard deviation from the means. Data were subjected to an analysis of variance (ANOVA) by using both Statistica version 10 (Statsoft, USA) and Statgraphics® (StatPoint Technologies Inc., USA). Significant differences were expressed using 90% and 95% confidence intervals. A 90% Confidence interval was used for vegetative growth due to the natural occurring variability in grapevine growth.

## 3.3 RESULTS AND DISCUSSION

### 3.3.1 Chemical composition of irrigation water

Winery wastewater usually contains higher concentrations of certain elements, which may affect grapevine performance and wine quality. Furthermore, the high Na and K levels in winery wastewater lead to elevated EC and SAR values. Results for chemical composition of the various irrigation water treatments are presented in Table 3.1.

The heavy metal content of the irrigation water was not affected by the ratio of raw river water to raw winery wastewater in the irrigation treatments. Furthermore, the amount of heavy metals that were present was negligible for all irrigation treatments.

The only elements and ions that increased as the level of COD in the augmented winery wastewater increased were P, K, Na, Ca, bicarbonate ($HCO_3^-$) and B. Therefore, these were the only ones that may have resulted in variation in grapevine performance and/or wine quality due to irrigation using different levels of augmented winery wastewater. The amount of P that was applied through the various irrigation treatments increased as the level of COD in the augmented winery wastewater increased. As there are no guidelines for P levels in irrigation water (Department of Water Affairs and Forestry, 1996; ANZECC, 2000), the amount applied through irrigation using winery wastewater are legally acceptable and may even reduce the need for P fertilization.

There was a large increase in the amount of K and Na that were applied through the irrigation water, associated with an increase in the level of COD in the augmented winery wastewater. Therefore, a high correlation was observed between the amount of Na and K applied and the level of COD in the irrigation water (Figs. 3.2 – 3.5). Potassium and Na are the most important cations which may affect grapevine performance and wine quality as discussed earlier; therefore, it is important that water quality is monitored in terms of these cations before wastewater irrigation is considered. The amount of Ca applied also increased with an increase in the level of COD in the irrigation water. There is no guideline for recommended Ca in water, thus, the amount applied through the various irrigation treatments should not have a negative impact on the irrigated grapevines.

**Table 3.1** Total calculated amounts of phosphorus, cations, anions, boron and heavy metals applied *via* raw river water and augmented winery wastewater used for irrigation of Cabernet Sauvignon/99R in the 2010/11 and 2011/12 seasons.

| Treatment no. | Target COD (mg/L) | EC* | SAR* | P | N ($NO_3^-$ +$NH_4^+$) | K | Na | Ca | Mg | Fe | Cl | $HCO_3^-$ | $SO_4$ | B | Cd | Cr | As |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **Amount applied in 2010/11 (kg/L)** | | | | | | | | | | | | | | | |
| T1 | Raw water | 11.00 | 0.50 | 0.09 | 5.53 | 8.0 | 52.9 | 31.70 | 20.10 | 1.29 | 122.5 | 79.9 | 114.2 | 0.08 | 0.00 | 0.09 | 0.00 |
| T2 | 100 | 11.05 | 0.50 | 0.07 | 3.61 | 13.5 | 53.6 | 30.70 | 19.60 | 2.12 | 116.1 | 79.8 | 110.1 | 0.10 | 0.00 | 0.11 | 0.00 |
| T3 | 250 | 11.94 | 0.57 | 0.39 | 3.28 | 25.1 | 59.8 | 30.80 | 19.80 | 1.88 | 118.2 | 93.1 | 112.8 | 0.13 | 0.00 | 0.10 | 0.00 |
| T4 | 500 | 13.16 | 0.60 | 1.18 | 2.62 | 43.9 | 73.1 | 32.90 | 20.20 | 1.95 | 115.2 | 113.6 | 121.2 | 0.18 | 0.00 | 0.09 | 0.10 |
| T5 | 1000 | 15.99 | 0.71 | 3.18 | 3.01 | 79.0 | 81.2 | 34.90 | 20.70 | 1.17 | 117.4 | 144.3 | 111.4 | 0.29 | 0.00 | 0.10 | 0.00 |
| T6 | 1500 | 18.43 | 0.82 | 5.35 | 4.29 | 112.8 | 97.3 | 38.50 | 21.60 | 2.24 | 122.6 | 253.2 | 105.1 | 0.39 | 0.00 | 0.11 | 0.00 |
| T7 | 2000 | 20.99 | 0.87 | 8.46 | 4.76 | 148.8 | 104.7 | 39.90 | 22.20 | 1.95 | 124.1 | 240.4 | 114.0 | 0.49 | 0.00 | 0.08 | 0.00 |
| T8 | 2500 | 22.71 | 0.88 | 10.37 | 4.69 | 173.4 | 110.1 | 40.80 | 22.10 | 2.64 | 121.6 | 267.8 | 103.0 | 0.58 | 0.00 | 0.09 | 0.00 |
| T9 | 3000 | 25.08 | 1.01 | 13.46 | 5.43 | 214.8 | 122.3 | 43.10 | 22.90 | 3.29 | 127.7 | 355.4 | 138.5 | 0.71 | 0.00 | 0.09 | 0.00 |
| | | **Amount applied in 2011/12 (kg/L)** | | | | | | | | | | | | | | | |
| T1 | Raw water | 7.40 | 0.51 | 0.07 | 3.32 | 6.6 | 32.8 | 14.90 | 9.70 | 0.20 | 67.4 | 36.1 | 55.1 | 0.03 | 0.01 | 0.01 | 0.01 |
| T2 | 100 | 8.05 | 0.54 | 0.23 | 1.47 | 11.0 | 35.5 | 15.40 | 9.90 | 0.30 | 70.2 | 40.9 | 52.0 | 0.03 | 0.00 | 0.01 | 0.01 |
| T3 | 250 | 9.19 | 0.55 | 0.63 | 1.25 | 21.6 | 37.5 | 17.50 | 10.40 | 0.40 | 68.5 | 54.2 | 53.9 | 0.05 | 0.00 | 0.01 | 0.00 |
| T4 | 500 | 10.94 | 0.60 | 1.37 | 2.02 | 35.9 | 42.4 | 20.10 | 10.60 | 0.60 | 67.5 | 70.7 | 53.7 | 0.10 | 0.00 | 0.01 | 0.01 |
| T5 | 1000 | 13.84 | 0.64 | 2.95 | 2.22 | 65.1 | 49.5 | 25.00 | 11.30 | 0.90 | 66.5 | 100.7 | 102.8 | 0.17 | 0.01 | 0.01 | 0.01 |
| T6 | 1500 | 16.17 | 0.68 | 4.43 | 3.09 | 87.3 | 54.9 | 28.60 | 12.20 | 1.30 | 68.6 | 137.4 | 77.4 | 0.24 | 0.01 | 0.01 | 0.00 |
| T7 | 2000 | 17.84 | 0.70 | 5.24 | 4.26 | 104.0 | 61.5 | 32.50 | 12.40 | 1.50 | 63.2 | 160.9 | 142.4 | 0.32 | 0.00 | 0.01 | 0.00 |
| T8 | 2500 | 21.14 | 0.76 | 6.82 | 6.31 | 134.5 | 69.1 | 36.50 | 12.80 | 2.10 | 78.2 | 177.6 | 148.1 | 0.41 | 0.00 | 0.01 | 0.00 |
| T9 | 3000 | 23.04 | 0.79 | 8.03 | 6.4 | 157.7 | 74.0 | 40.70 | 13.70 | 2.60 | 78.8 | 227.3 | 129.5 | 0.44 | 0.00 | 0.01 | 0.00 |

*EC = Electrical conductivity; SAR = Sodium adsorption ratio

Still, it is important to calculate the Ca concentration in order to determine the SAR of the water (Myburgh, 2012). When the Ca to Mg ratio in water is less than one, the potential effect that Na may have is increased (Ayers and Westcott, 1985). An increase was observed in the amount of $HCO_3^-$ applied *via* irrigation water as the level of COD in the augmented winery wastewater increased. Even though no recommended guidelines exist for $HCO_3^-$ in irrigation water (Department of Water Affairs and Forestry, 1996; ANZECC, 2000), high levels can affect plants, soil and irrigation equipment. Furthermore, irrigation using water rich in $HCO_3^-$ results in the precipitation of Ca and Mg in the soil which results in an increase in relative Na (Van Zyl, 1981; McCarthy et al., 1992; Scherer *et al*., 1996). This increase causes an increase the SAR which may result in a decline in soil physical properties (Van Zyl, 1981; ANZECC, 2000). When irrigation is applied with $HCO_3^-$ rich water, soil pH may increase which restricts ion uptake by plants (ANZECC, 2000). Therefore, it is very important to monitor the $HCO_3^-$ concentration when irrigating augmented winery wastewater.

Although larger quantities of B was applied by the more concentrated wastewater irrigation treatments, the B concentration very seldom exceeded 0.50 mg/L which is classified as suitable for grapevines (McCarthy *et al.,* 1992). Furthermore, it rarely ever exceeded 0.75 mg/L, the maximum recommended value by Ayers and Westcott (1985), for any of the irrigation treatments. It is, however, very important to monitor B levels when irrigating augmented winery wastewater as grapevine have been classified as very sensitive (Van Zyl, 1981) and sensitive (Ayers & Westcott, 1985; Department of Water Affairs and Forestry, 1996; ANZECC, 2000) to B toxicity.

Due to the elevated K and Na levels, the EC and SAR also increased as the level of COD in the augmented winery wastewater increased. Still, average values for all wastewater irrigation treatments were comfortably within the legal requirements for EC and SAR for irrigation water, which is <200 mS/m and <5 respectively (Department of Water Affairs and Forestry, 2004). These values should be monitored carefully when wastewater irrigation is considered. High SAR values reflect on high Na levels which may cause soil structural degradation, soil compaction and soil sodicity (Department of Water Affairs and Forestry, 1996). Electrical conductivity indicates the amount of dissolved salts in the water. Therefore, high EC levels indicate the presence of high salt concentrations in the water which may accumulate in the soil.

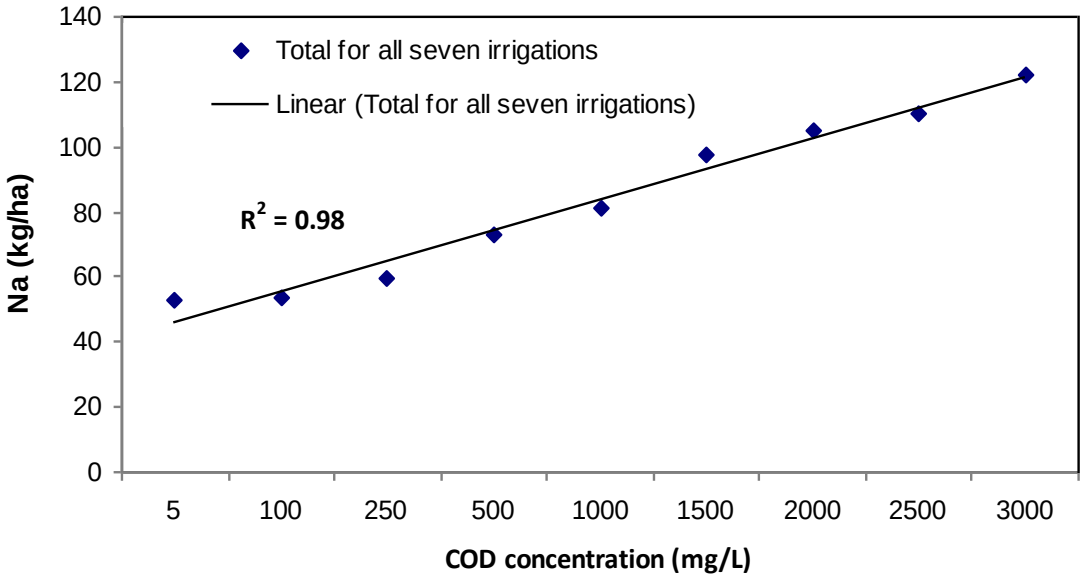


**Fig 3.2** The correlation between amount of sodium (Na) applied *via* the various irrigation treatments and the COD concentration in the irrigation water during the 2010/11 season.

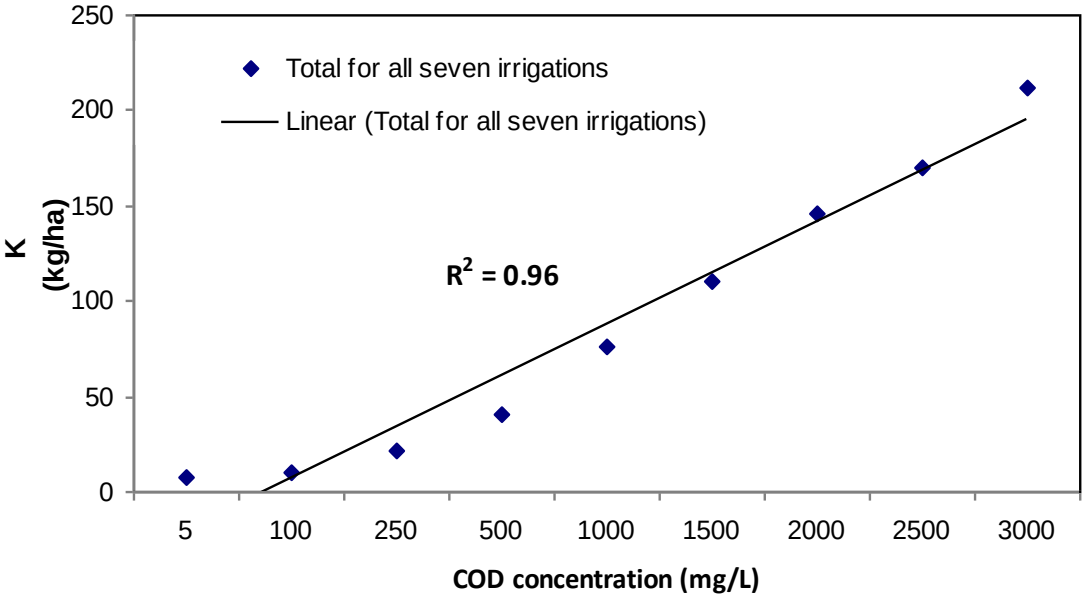


**Fig 3.3** The correlation between amount of potassium (K) applied *via* the various irrigation treatments and the COD concentration in the irrigation water during the 2010/11 season.

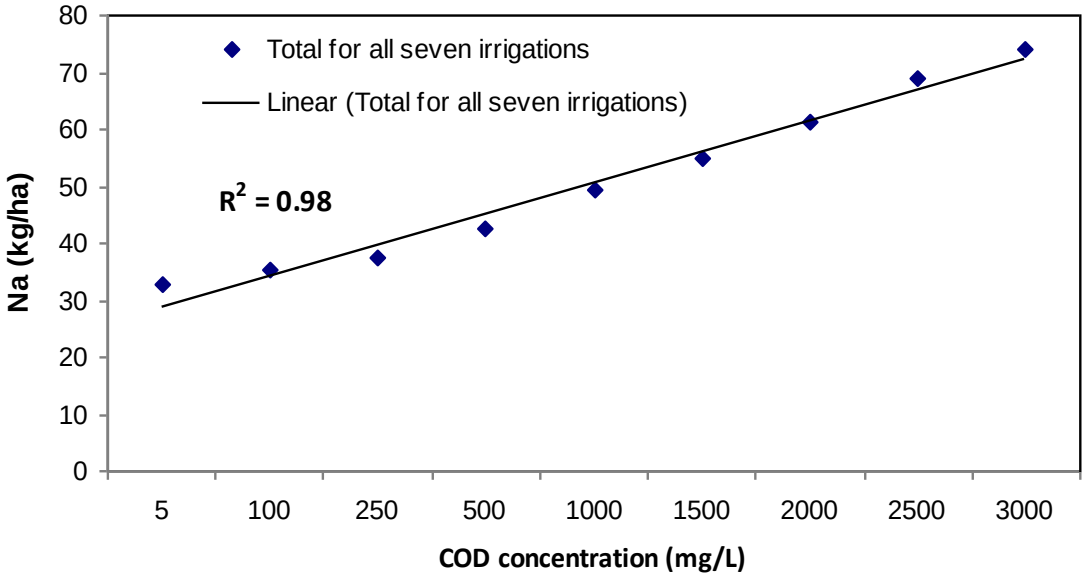


**Fig 3.4** The correlation between amount of sodium (Na) applied *via* the various irrigation treatments and the COD concentration in the irrigation water during the 2011/12 season.

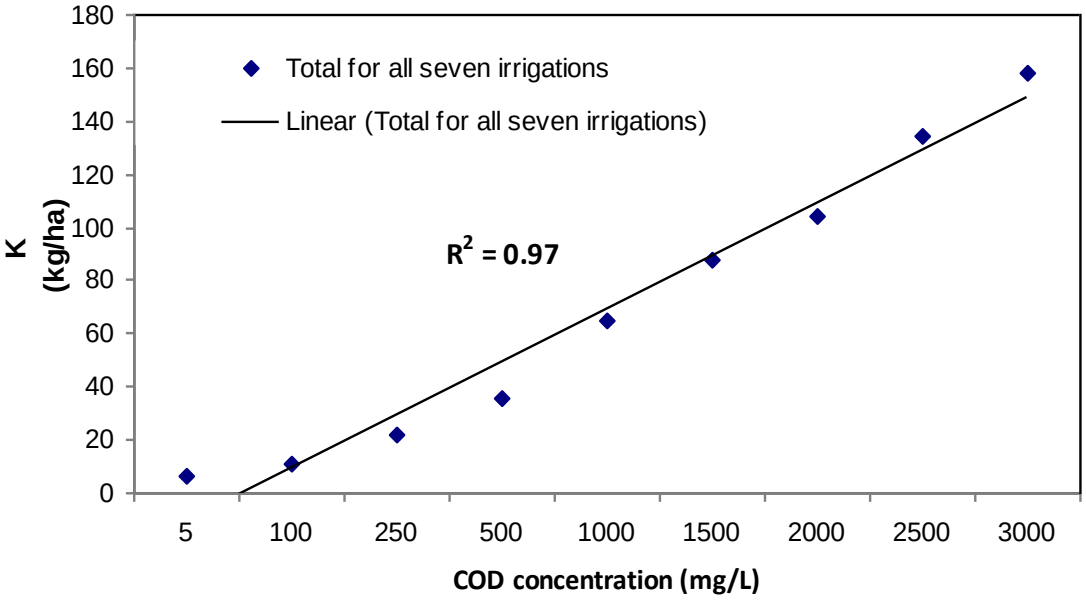


**Fig 3.5** The correlation between amount of potassium (K) applied *via* the various irrigation treatments and the COD concentration in the irrigation water during the 2011/12 season.

### 3.3.2 Microbial composition of irrigation water

Winery wastewater contains large populations of micro-organisms, mainly belonging to yeast and bacterial species that naturally occur in wine (Malandra *et al.,* 2003; Jourjon *et al.,* 2005). The lowest yeast cell populations were present in the raw irrigation water, containing less than

$10^3$ colony forming units per millilitre (cfu/mL) during three of the four irrigations periods (Fig 3.6). The highest yeast cell population were observed in the raw winery wastewater, ranging from 2.48 x $10^6$ to 8.72 x $10^6$. Furthermore, the target COD levels for the various treatments were obtained by augmenting the raw winery wastewater, containing 25000, 15000, 6000 and 7000 mg/L COD respectively, with raw river water. Therefore, total yeast population in irrigation water showed a clear tendency to increase as the level of COD in the augmented winery wastewater increased.

As yeasts form an integral part of the winemaking process, large yeast cell populations were to be expected. The total microbial flora of raw winery wastewater, as well as irrigation treatments containing a significant fraction raw winery wastewater, was dominated by the presence of yeast cells. Furthermore, as *Saccharomyces cerevisiae* is usually the dominant yeast strain found during winemaking, it probably accounted for the majority of the yeast population. However, various yeast species, including *Saccharomyces cerevisiae, Candida intermedia, Hanseniaspora uvarum* and *Pichia membranaefaciens*, have been found to be present in winery wastewater (Coetzee *et al.,* 2004; Jourjon *et al.,* 2005). The identification of yeast cell species was beyond the scope of this study, thus, determining which species dominated and which were present in fewer numbers are not discussed. If these yeast cells are transferred into grape juice and wine, it may affect alcoholic fermentation and wine composition and quality to a certain extent. Furthermore, as greater numbers would probably be transferred from the irrigation treatments containing larger cell populations, a bigger impact would be observed on wine composition and quality.

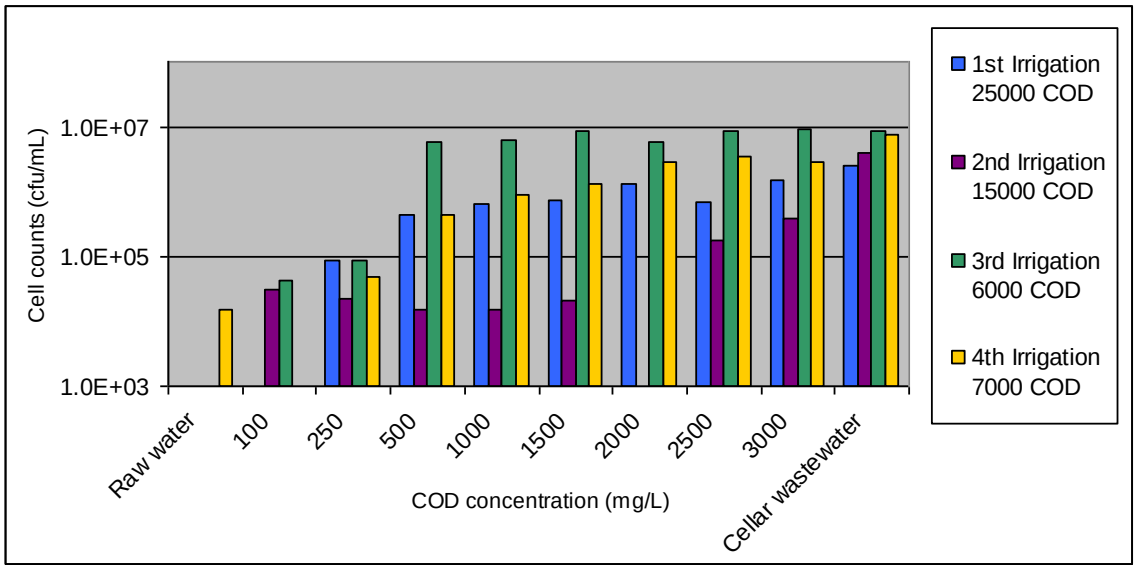


**Fig 3.6** Total yeast cell counts (cfu/mL) for the various irrigation water treatments for all four irrigations applied during the 2011/12 season.

The total bacterial flora, present in the various irrigation treatments, did not seem to be dependent on the ratio of winery wastewater to raw water (Fig 3.7). This could be explained by the fact that winery wastewater did not necessarily contain higher bacterial numbers than raw water. Therefore, as bacterial populations did not vary in accordance with the various irrigation treatments, any variation in must or wine bacterial flora would not be associated with a wastewater irrigation treatment effect. Total bacterial numbers ranged from 1.1 x $10^3$ cfu/mL to 8.8 x $10^5$ cfu/mL.

When compared to yeast cell populations, bacteria cells made a minor contribution to the total microbial flora of the wastewater irrigation treatments which contained a large volume of winery wastewater. On the contrary, bacteria species overwhelmingly dominated the microbial flora of the raw water and treatments containing small volumes of winery wastewater as low yeast cell numbers were present for these treatments. As identification of the various bacteria species was beyond the scope of this study, the dominating genera and strains are not discussed. Figure A1 in Addendum A illustrate the increase in total microbial flora, due to an increase in yeast numbers, associated with a decrease in the level of augmentation of winery wastewater.

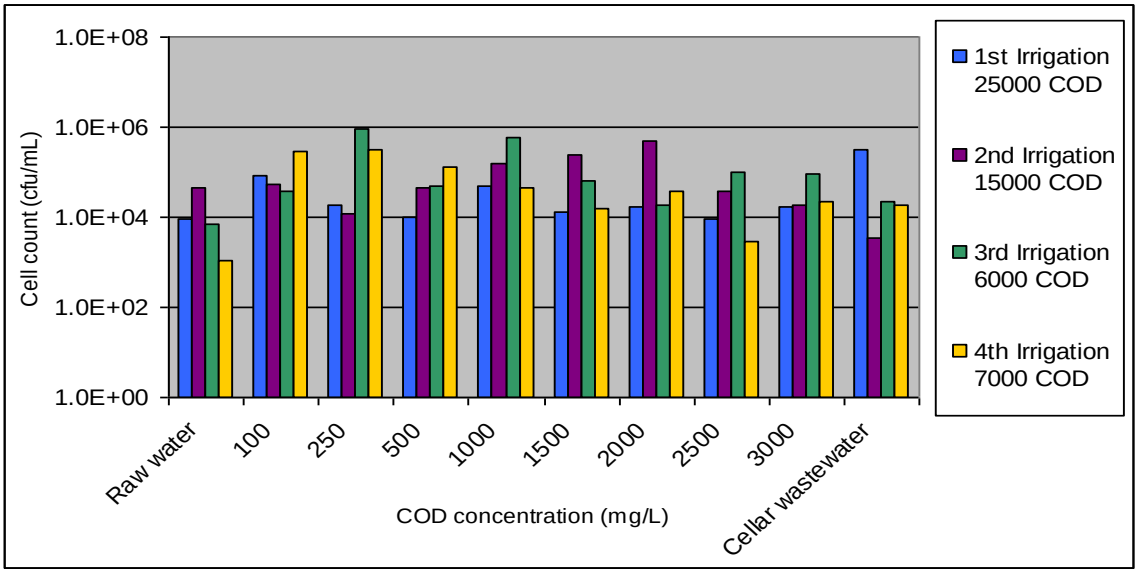


**Fig 3.7** Total bacterial counts (cfu/mL) for the various irrigation water treatments for all four irrigations applied during the 2011/12 season.

Large variations in microbial composition were observed between the four periods of irrigation. Variation in the yeast micro flora was especially apparent, while bacterial counts did not vary as much. These variations illustrate the diversity which occurs in the microbial composition of winery wastewater during different periods of the season. Furthermore, the higher the COD of the winery wastewater before augmentation were, the lower the total microbial population tended to be (Fig 3.8).

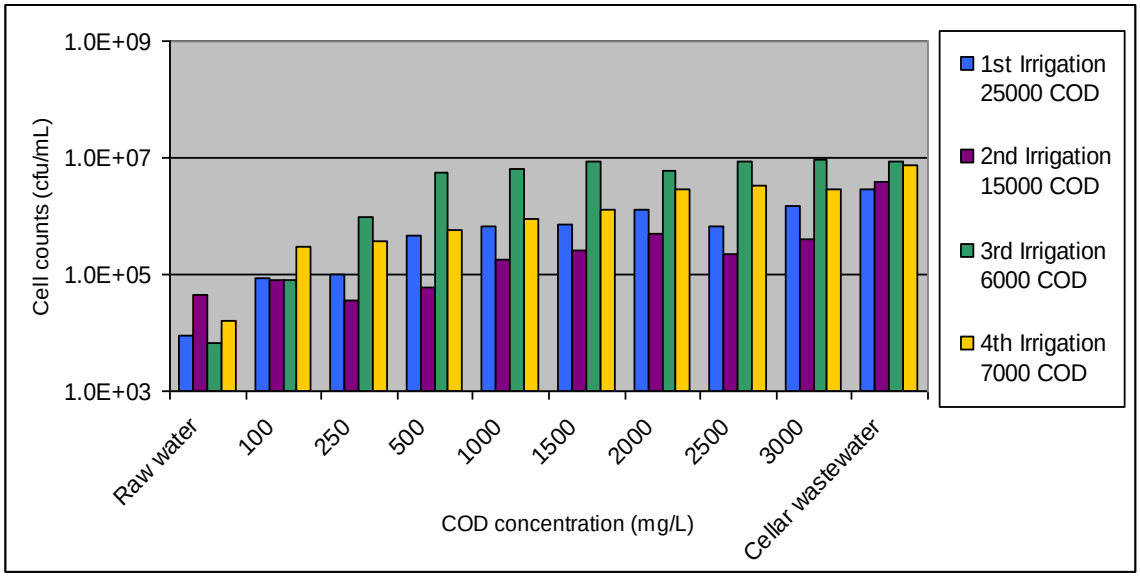


**Fig 3.8** Total microbial counts (cfu/mL) for the various irrigation water treatments for all four irrigations applied during the 2011/12 season.

Total microbial numbers for irrigation water from the Holsloot River was within ranges found for South African rivers (Fig 3.8) (Paulse *et al.,* 2009). Total bacterial counts also compared with counts for several other South African rivers (Kinge and Mbewe, 2012). In addition, winery wastewater contained similar microbial populations as found by Jourjon *et al.* (2005). Yeast populations, however, exceeded those found by Malandra *et al*. (2003), while bacterial counts were significantly less. These similarities indicate that the microbial composition of the various irrigation treatments represented the microbial populations which would most likely be found in augmented winery wastewater.

### 3.3.3 Vegetative growth

Grapevines were subjected to similar climatic and soil conditions and received identical amounts of irrigation water and fertilization throughout the season. It may therefore be assumed that any variation in vegetative growth could not be attributed to a wastewater irrigation treatment effect. No differences in any of the vegetative growth parameters between any of the treatments were observed at a 95% confidence level. Interpretation of the data at a 90% level still revealed no differences in either the 2010/11 or the 2011/12 season (Table 3.2).

Primary shoot length was similar for all treatments in both seasons. Secondary shoot length showed considerable variability between treatments in the 2010/11 season at a 90% confidence level, but none of the wastewater irrigation treatments had any effect on secondary shoot length when compared to the raw water irrigation control. The variability in secondary shoot length could therefore not be attributed to a treatment effect, but rather to variation in primary shoot growth vigour at the time of topping as well as the timing and severity of topping, stimulating

lateral shoot growth. Mean primary and secondary shoot length decreased slightly from the 2010/11 to the 2011/12 season. For most treatments, primary and secondary shoot leaf area decreased considerably from the 2010/11 to the 2011/12 season. This decrease was probably caused by a decrease in number of leaves in the 2011/12 season, resulting from less vigour, the timing of topping, shorter topping and better exposure of the leaves.

The various wastewater irrigation treatments did not affect cane mass at pruning. Cane mass was considerably higher in the 2011/12 season than in the 2010/11 season, probably as a result of an increase in the number of shoots per vine and/or an increase in shoot thickness. Thus, vigour per shoot was less in the 2011/12 season, but due to an increase in number of shoots (bud load), total growth per grapevine was higher. Decreased growth per shoot in the 2011/12 season may be associated with cooler climatic conditions during this season. Shoots per vine and shoot thickness were, however, not measured and therefore no definite conclusions can be made. Furthermore, the increase in bunches per vine and yield in the 2011/12 season corresponds with an increase in number of shoots per vine. In general, the results indicate that vegetative growth was not affected by any of the wastewater irrigation treatments.

To our knowledge, no previous research had been done on the effect of winery wastewater irrigation on grapevine vegetative growth. However, raw and diluted winery wastewater has been found to inhibit vegetative growth in barley, millet, lucerne and phalaris (Mosse *et al.,* 2010). Furthermore, if the osmotic potential of the soil solution is decreased as a result of the larger amounts of salts that are applied through wastewater irrigation, it may impede with grapevine transpiration and photosynthesis, causing a decrease in shoot growth (Greenway and Munns, 1980; Munns and Termaat, 1986; Volkmar *et al.,* 1998; Shannon and Grieve, 1999; Munns, 2002). The reason that grapevine growth was not affected in this study is probably due to higher quality wastewater and the high tolerance of grapevines to phytotoxicity when compared to these crops. In addition, plant water status, and therefore transpiration and photosynthesis, was not affected by irrigation using augmented winery wastewater. Grapevine water status was determined by means of predawn ($\Psi_{PD}$), as well as midday leaf ($\Psi_{L}$) and stem ($\Psi_{S}$) water potential measurements on 8 December and 16 March (data not shown). It may be possible that water uptake by the grapevines and subsequent grapevine growth may have been restricted by wastewater irrigation if a different, less tolerable, rootstock was used. Furthermore, the nutrient that generally stimulates vegetative growth, namely nitrogen, was not applied in large amounts *via* any of the wastewater irrigation treatments in this study (Table 3.1).

**Table 3.2** Shoot length and leaf area of Cabernet Sauvignon/99R grapevines irrigated using augmented winery wastewater in the 2010/11 and 2011/12 seasons, as well as cane mass at pruning in July (each value represents the average of triplicate treatments).

| Treatment no. | Target COD (mg/L) | 2011 | | | | | | 2012 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Primary shoot length (cm) | secondary shoot length (cm) | Number of leaves per primary shoot | Primary shoot leaf area ($cm^2$) | Secondary shoot leaf area ($cm^2$) | Cane mass (t/ha) | Primary shoot length (cm) | secondary shoot length (cm) | Number of leaves per primary shoot | Primary shoot leaf area ($cm^2$) | Secondary shoot leaf area ($cm^2$) | Cane mass (t/ha) |
| T1 | Raw water | 80.8 a[(1)] ± 3.2 | 39.3 abc ± 22.8 | 15.4 a ± 4.0 | 1477.9 a ± 358 | 805.9 a ± 447 | 2.3 a ± 0.4 | 86.7 a ± 11.1 | 31.8 a ± 6.2 | 14.8 a ± 3.8 | 1435.9 a ± 423 | 576.8 a ± 147 | 2.7 a ± 0.7 |
| T2 | 100 | 106.3 a ± 5.3 | 57.3 ab ± 18.0 | 17.0 a ± 1.9 | 1713.7 a ± 97 | 1206.7 a ± 369 | 2.5 a ± 0.7 | 77.3 a ± 5.3 | 35.9 a ± 17.0 | 10.9 a ± 1.7 | 971.9 a ± 83 | 679.3 a ± 430 | 2.8 a ± 0.9 |
| T3 | 250 | 102.1 a ± 6.2 | 62.9 a ± 23.6 | 16.8 a ± 2.0 | 1762.2 a ± 287 | 902.9 a ± 230 | 2.4 a ± 0.1 | 78.8 a ± 6.2 | 37.9 a ± 6.5 | 13.1 a ± 2.5 | 1256.5 a ± 88 | 726.3 a ± 207 | 2.7 a ± 0.5 |
| T4 | 500 | 99.1 a ± 15.3 | 26.7 bc ± 1.9 | 15.3 a ± 1.6 | 1576.7 a ± 193 | 546.9 a ± 80 | 2.4 a ± 0.7 | 94.8 a ± 15.3 | 41.9 a ± 25.6 | 14.3 a ± 3.1 | 1368.7 a ± 394 | 864.1 a ± 495 | 2.8 a ± 0.8 |
| T5 | 1000 | 88.9 a ± 3.2 | 31.5 abc ± 18.7 | 14.8 a ± 2.5 | 1484.6 a ± 127 | 724.6 a ± 352 | 2.2 a ± 0.5 | 96.3 a ± 13.0 | 29.6 a ± 11.1 | 15.1 a ± 0.9 | 1291.8 a ± 30 | 586.3 a ± 204 | 2.6 a ± 0.8 |
| T6 | 1500 | 100.4 a ± 10.6 | 47.2 abc ± 17.5 | 17.2 a ± 1.9 | 1781.4 a ± 193 | 973.2 a ± 256 | 1.9 a ± 0.6 | 85.4 a ± 10.6 | 33.3 a ± 16.2 | 14.1 a ± 4.8 | 1199.5 a ± 300 | 538.8 a ± 168 | 2.5 a ± 0.6 |
| T7 | 2000 | 88.3 a ± 4.9 | 34.4 bc ± 13.3 | 14.8 a ± 2.2 | 1391.5 a ± 60 | 779.1 a ± 370 | 2.0 a ± 0.1 | 87.0 a ± 4.9 | 34.9 a ± 5.1 | 13.8 a ± 3.0 | 1176.0 a ± 142 | 547.1 a ± 115 | 2.4 a ± 0.2 |
| T8 | 2500 | 93.9 a ± 5.9 | 16.5 c ± 3.2 | 18.4 a ± 1.4 | 1644.3 a ± 42 | 503.1 a ± 75 | 2.3 a ± 0.4 | 94.6 a ± 5.9 | 57.5 a ± 10.5 | 13.6 a ± 3.0 | 1430.2 a ± 122 | 868.5 a ± 212 | 2.5 a ± 0.3 |
| T9 | 3000 | 89.9 a ±20.7 | 43.7 abc ± 34.8 | 18.8 a ± 1.0 | 1843.5 a ± 206 | 988.8 a ± 576 | 2.2 a ± 0.4 | 84.9 a ± 20.7 | 34.5 a ± 14.8 | 13.7 a ± 2.5 | 1171.7 a ± 184 | 619.2 a ± 210 | 2.8 a ± 0.5 |
| | | 94.4 | 39.9 | 16.5 | 1630.6 | 825.7 | 2.2 | 87.3 | 37.5 | 13.7 | 1255.8 | 667.4 | 2.6 |

[(1)] Values designated by the same letters within a column do not differ significantly ($p \leq 0.10$)

± Values indicate standard deviation from the mean

### 3.3.4 Berry development and composition

3.3.4.1 Berry mass and volume

Increases in berry mass and volume indicated a double sigmoid curve (Figs. 3.9 – 3.12) as normally observed (Coombe, 1992). This would have been more clearly visible if berry sampling started earlier. A decrease in berry mass and volume was observed during the later stages of ripening in the 2010/11 season. This decrease may be due to water loss by the berry which occurs due to a decrease in water transport to the berry and a simultaneous continuation of water loss from the berry caused by transpiration (Coombe, 1992). Cooler climatic conditions in the 2011/12 season resulted in delayed berry development compared to the 2010/11 season. The pattern and rate of berry development were similar for all treatments monitored (T1, T5, T7 & T9). The results therefore show that the selected wastewater irrigation treatments had no effect on berry size when compared to raw water irrigation throughout berry development and ripening in either of the two seasons (Figs. 3.9 – 3.12). Measurements on all nine wastewater irrigation treatments at harvest in the 2010/11 season indicated that T2 had a higher berry mass than T9 (Table 3.3). However, since no differences in grapevine water status were found, the occurrence of smaller berries in T9 is inexplicable. In the 2011/12 season no variation in berry size occurred between any of the irrigation treatments.

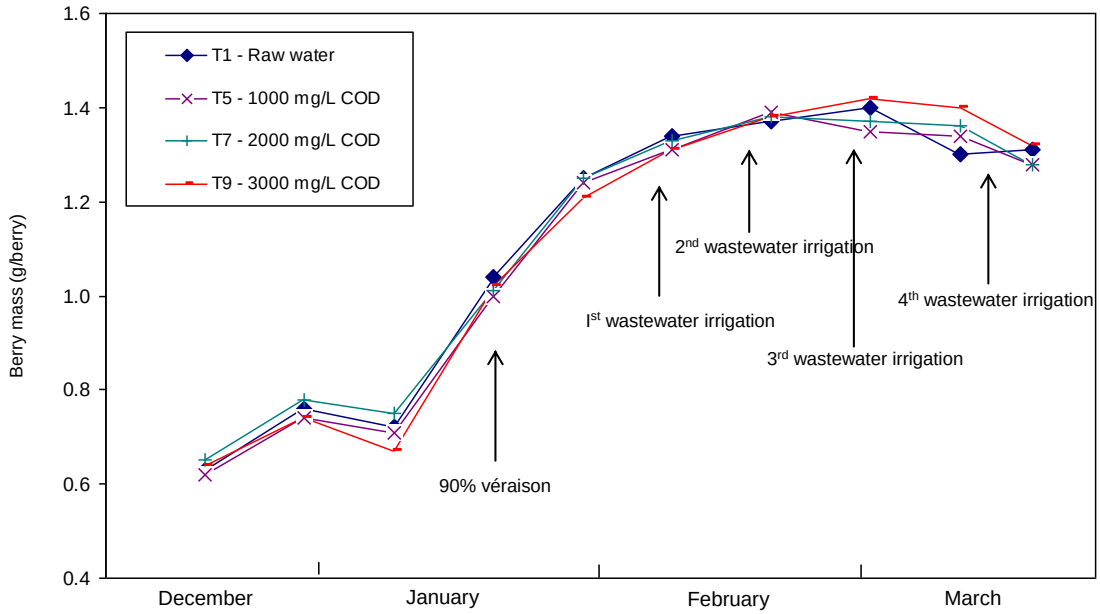


**Fig 3.9** The effect of irrigation using augmented winery wastewater on berry mass of Cabernet Sauvignon/99R grapevines during ripening in the 2010/11 season. Indicated values represent the average of triplicate treatments (standard deviation not shown).

**Table 3.3** Yield components of Cabernet Sauvignon/99R grapevines irrigated using augmented winery wastewater during the 2010/11 and 2011/12 seasons (each value represents the average of triplicate treatments)**.**

| Treatment no. | Target COD (mg/L) | 2010/11 | | | | | 2011/12 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Bunches per grapevine | Berry mass (g) | Bunch mass (g) | Berry volume ($cm^3$) | Yield (t/ha) | Bunches per grapevine | Berry mass (g) | Bunch mass (g) | Berry volume ($cm^3$) | Yield (t/ha) |
| T1 | Raw water | 23 ab[(1)] ± 0.3 | 1.31 ab ± 0.04 | 126 a ± 16.2 | 1.20 a ± 0.03 | 9.8 abc ± 1.4 | 33 a ± 5.0 | 1.36 a ± 0.09 | 153 a ± 18.0 | 1.25 a ± 0.09 | 17.2 a ± 2.2 |
| T2 | 100 | 24 a ± 4.1 | 1.38 a ± 0.05 | 135 a ± 16.4 | 1.27 a ± 0.04 | 11.2 a ± 0.6 | 31 a ± 1.2 | 1.38 a ± 0.05 | 156 a ± 8.1 | 1.27 a ± 0.04 | 17.0 a ± 1.5 |
| T3 | 250 | 23 ab ± 1.3 | 1.34 ab ± 0.06 | 139 a ± 3.5 | 1.23 a ± 0.06 | 11.0 ab ± 0.5 | 32 a ± 0.4 | 1.31 a ± 0.10 | 155 a ± 5.3 | 1.22 a ± 0.09 | 17.4 a ± 0.7 |
| T4 | 500 | 22 ab ± 1.4 | 1.31 ab ± 0.09 | 139 a ± 10.4 | 1.20 a ± 0.08 | 10.6 ab ± 0.7 | 32 a ± 5.1 | 1.34 a ± 0.01 | 163 a ± 1.7 | 1.24 a ± 0.01 | 18.0 a ± 2.9 |
| T5 | 1000 | 24 a ± 3.1 | 1.28 ab ± 0.02 | 126 a ± 6.4 | 1.18 a ± 0.01 | 10.6 ab ± 1.7 | 33 a ± 1.0 | 1.33 a ± 0.07 | 152 a ± 5.7 | 1.25 a ± 0.05 | 17.5 a ± 0.2 |
| T6 | 1500 | 23 ab ± 2.4 | 1.36 ab ± 0.06 | 136 a ± 18.5 | 1.26 a ± 0.06 | 10.6 ab ± 0.9 | 30 a ± 3.7 | 1.36 a ± 0.06 | 165 a ± 15.8 | 1.27 a ± 0.06 | 16.9 a ± 1.0 |
| T7 | 2000 | 19 b ± 1.2 | 1.32 ab ± 0.06 | 123 a ± 2.3 | 1.22 a ± 0.06 | 8.2 c ± 0.6 | 32 a ± 0.9 | 1.36 a ± 0.01 | 150 a ± 18.1 | 1.26 a ± 0.01 | 16.6 a ± 2.5 |
| T8 | 2500 | 24 a ± 5.3 | 1.36 ab ± 0.09 | 134 a ± 14.7 | 1.26 a ± 0.08 | 11.2 a ± 1.7 | 31 a ± 2.2 | 1.41 a ± 0.07 | 173 a ± 9.8 | 1.29 a ± 0.06 | 18.6 a ± 0.3 |
| T9 | 3000 | 23 ab ± 0.3 | 1.27 b ± 0.05 | 119 a ± 5.5 | 1.16 a ± 0.04 | 9.4 bc ± 0.4 | 31 a ± 4.5 | 1.35 a ± 0.06 | 142 a ± 3.4 | 1.24 a ± 0.04 | 16.5 a ± 0.4 |

[(1)] Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$)

± Values indicate standard deviation from the mean

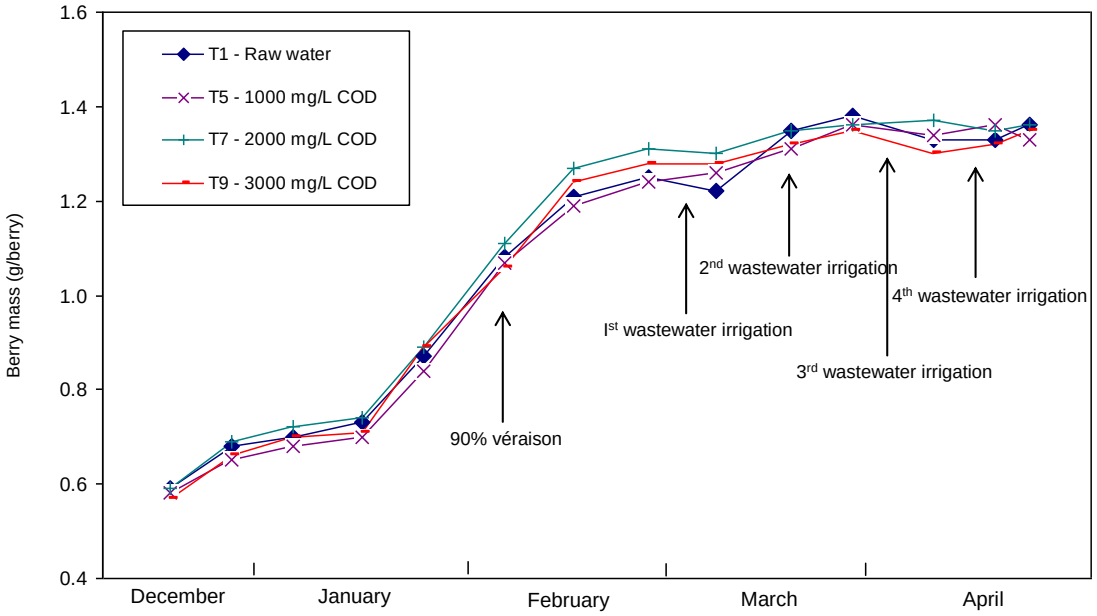


**Fig 3.10** The effect of irrigation using augmented winery wastewater on berry mass of Cabernet Sauvignon/99R grapevines during ripening in the 2011/12 season. Indicated values represent the average of triplicate treatments (standard deviation not shown).

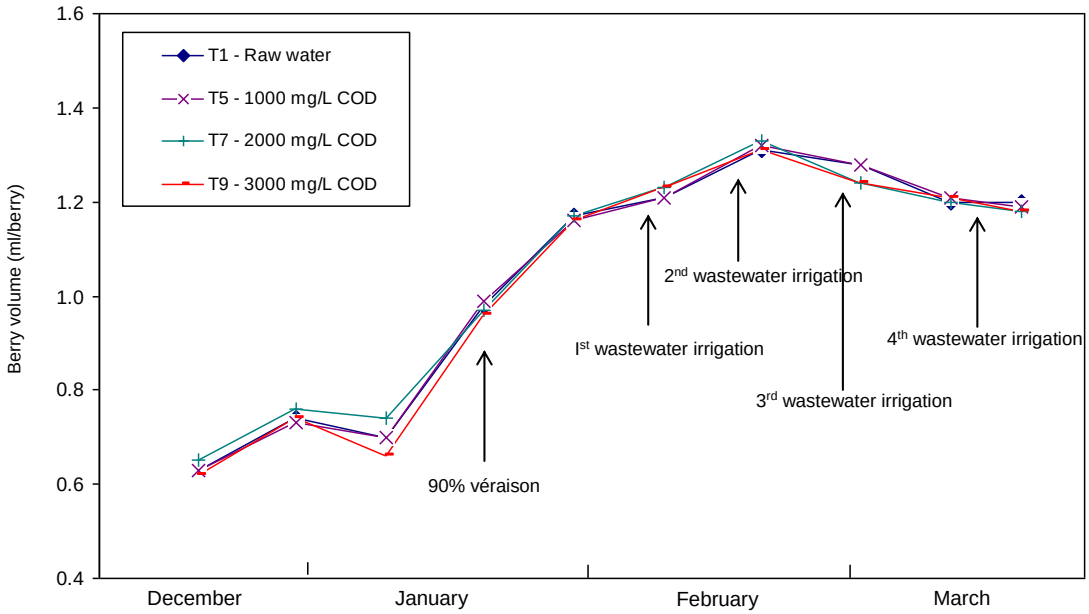


**Fig 3.11** The effect of irrigation using augmented winery wastewater on berry volume of Cabernet Sauvignon/99R grapevines during ripening in the 2010/11 season. Indicated values represent the average of triplicate treatments (standard deviation not shown).

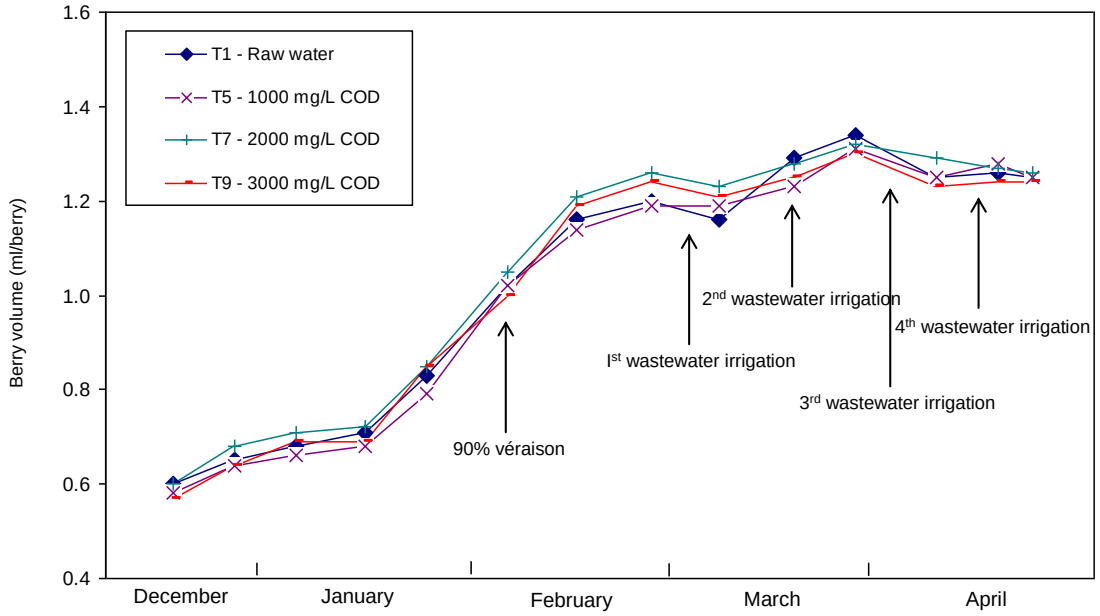


**Fig 3.12** The effect of irrigation using augmented winery wastewater on berry volume of Cabernet Sauvignon/99R grapevines during ripening in the 2011/12 season. Indicated values represent the average of triplicate treatments (standard deviation not shown).

### 3.3.4.2 Juice characteristics

#### 3.3.4.2.1 Nitrogen, phosphorus, cations and heavy metals

Juice N, P and cation content for all irrigation treatments were within the norms for wine grapes (Saayman, 1981; Ough and Kriel, 1985; Haight and Gump, 1995; Conradie, 2001; Myburgh, 2006; Lategan, 2011). There were no differences in juice P, K and Mg concentration in either of the two seasons (Table 3.4). The amount of P and K applied through the various irrigation treatments increased greatly with an increase in level of COD of the augmented winery wastewater (Table 3.1). Additionally, when irrigation with K-rich water is applied, it often leads to elevated K levels in plant tissue (McCarthy, 1981; Neilsen *et al.,* 1989a). The foregoing suggests that it is possible that similar amounts of these elements were absorbed by grapevines from all treatments. Another possibility is that these elements are trapped in the berry skins and therefore is not released into the juice during normal preparation procedures for juice analysis.

Although differences were observed in juice N concentration in the 2010/11 season, no trends were observed with respect to wastewater irrigation treatments. For instance, the two treatments that produced the highest juice N concentrations were the control treatment and the irrigation treatment with the highest COD level. In the 2011/12 season there were no differences in juice N. In the 2010/11 season juice Na tended to increase as the COD concentration of the augmented winery wastewater increased (Table 3.4). However, only T9 had significantly higher juice Na than the control treatment. Furthermore, no differences in juice Na were observed in the 2011/12 season. The total amount of Na applied through irrigation using winery wastewater

increased with an increase in the level of COD (Table 3.1). The total amount of Na applied to the soil through wastewater irrigation was, however, far greater in the 2010/11 season compared to the 2011/12 season. It has previously been reported that irrigation using Na rich water increases the Na levels in grapevine tissue (Samra, 1985; Stevens *et al.*, 2011). Higher salt concentrations in the water result in larger increases. The greater increase in amount of Na applied in the 2010/11 season may therefore have resulted in the increase in juice Na observed with an increase in level of COD of the augmented winery wastewater. If Na builds up in the soil and reaches a concentration where water uptake is suppressed, it will lead to salinity-induced water stress (Department of Water Affairs and Forestry, 1996). Furthermore, depending on the duration and severity of these conditions, crop growth and yield may decline. Nutritionists agree that excessive sodium intake is very unhealthy for humans and may cause high blood pressure. If irrigation using augmented winery wastewater increases juice and wine Na to excessive levels, it may lead to a human health risk. Juice Na for all treatments were, however, far below the maximum recommended Na levels by the International Organisation of Vine and Wine (OIV) and the South African Department of Water Affairs and Forestry, which is 60 mg/L and 100 mg/L, respectively (Department of Water Affairs and Forestry, 1996).

Juice Ca on the other hand decreased as the COD concentration and juice Na of the augmented winery wastewater increased in the 2010/11 season (Table 3.4). Sodic soil conditions may cause high concentrations of Na in grapevine tissue and an associated reduced Ca concentration (Samra, 1985; Stevens *et al*., 2011; Stevens *et al*., 2012). There were no differences in juice Ca concentration in the 2011/12 season. This may be attributed to the similar juice Na concentrations in the 2011/12 season. Although the amount of Ca applied *via* irrigation increased as the level of COD of augmented winery wastewater increased, these results indicate that there were no differences in the amount of Ca absorbed by grapevines. With the exception of K and Ca, all analysed elements increased from the 2010/11 to the 2011/12 season for all irrigation treatments. Juice K and Ca increased for some treatments and decreased for others, without particular trends.

Although differences were observed in must Cr concentrations between different wastewater irrigation treatments in the 2010/11 season, they could not be related to the level of augmentation (Table 3.5). With the exception of Cd for T7, none of the other heavy metals (Cd, As, Pb & Hg) were detected in the grape must (Table 3.5). The Cd value for T7 is however small enough that it may be ignored. These extremely low concentrations, or absence, of heavy metals was expected since they were present at very low concentrations, or not present at all, in the raw water or augmented winery wastewater. For this reason heavy metal analysis was not repeated in the 2011/12 season.

**Table 3.4** Nitrogen, phosphorus and cation content in must of Cabernet Sauvignon/99R grapevines irrigated using augmented winery wastewater during the 2010/11 and 2011/12 seasons (each value represents the average of triplicate treatments).

| Treatment no. | Target COD (mg/L) | 2010/11 | | | | | | 2011/12 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | N (mg/L) | P (mg/L) | Na (mg/L) | K (mg/L) | Ca (mg/L) | Mg (mg/L) | N (mg/L) | P (mg/L) | Na (mg/L) | K (mg/L) | Ca (mg/L) | Mg (mg/L) |
| T1 | Raw water | 202.0 ab[(1)] ± 18.7 | 135.5 a ± 7.8 | 7.6 bc ± 0.2 | 1625.8 a ± 80 | 47.2 ab ± 6.4 | 97.4 a ± 3.5 | 239.3 a ± 41.0 | 181.6 a ±22.0 | 22.2 a ± 0.7 | 1793.6 a ± 566 | 41.1 a ± 4.0 | 113.4 a ± 5.7 |
| T2 | 100 | 147.7 bc ± 43.1 | 140.9 a ± 19.6 | 7.7 bc ± 0.3 | 1780.5 a ± 192 | 49.4 a ± 1.4 | 101.7 a ± 11.6 | 246.3 a ± 19.5 | 201.6 a ± 16.2 | 22.6 a ± 0.7 | 1822.4 a ± 134 | 46.2 a ± 1.7 | 124.8 a ± 1.8 |
| T3 | 250 | 146.9 bc ± 20.1 | 129.6 a ± 4.4 | 7.1 c ± 0.3 | 1675.4 a ± 302 | 43.8 bc ± 0.6 | 94.0 a ± 2.8 | 240.3 a ± 36.9 | 187.4 a ± 16.5 | 21.2 a ± 2.5 | 1478.8 a ± 106 | 44.4 a ± 3.1 | 114.6 a ± 6.9 |
| T4 | 500 | 126.5 c ± 65.1 | 131.0 a ± 4.8 | 8.0 bc ± 0.2 | 1852.3 a ± 189 | 42.1 cd ± 2.9 | 101.7 a ± 2.8 | 234.7 a ± 30.1 | 182.1 a ± 13.6 | 23.4 a ± 1.5 | 1677.2 a ± 257 | 45.4 a ± 1.6 | 116.8 a ± 8.7 |
| T5 | 1000 | 122.5 c ± 21.3 | 131.3 a ± 7.5 | 8.7 ab ± 0.5 | 1904.9 a ± 220 | 38.2 de ± 0.6 | 96.6 a ± 0.4 | 223.0 a ± 27.5 | 191.7 a ± 14.1 | 22.6 a ± 0.5 | 1684.9 a ± 167 | 46.9 a ± 3.8 | 116.0 a ± 1.6 |
| T6 | 1500 | 136.2 c ± 12.9 | 124.3 a ± 0.7 | 7.9 bc ± 0.5 | 1759.0 a ± 101 | 34.3 e ± 1.9 | 92.2 a ± 4.9 | 214.3 a ± 32.0 | 187.9 a ± 5.7 | 22.9 a ± 1.2 | 1717.2 a ± 61 | 44.8 a ± 2.3 | 113.2 a ± 2.5 |
| T7 | 2000 | 135.3 c ± 66.8 | 145.8 a ± 6.7 | 8.4 ab ± 1.0 | 1894.0 a ± 299 | 35.7 e ± 0.8 | 100.2 a ± 7.8 | 221.7 a ± 28.0 | 214.3 a ± 24.7 | 22.5 a ± 3.2 | 1852.0 a ± 353 | 48.8 a ± 3.8 | 121.4 a ± 5.0 |
| T8 | 2500 | 137.3 c ± 21.1 | 135.7 a ± 12.9 | 8.5 ab ± 0.3 | 1925.6 a ± 138 | 37.6 de ± 2.3 | 96.0 a ± 6.6 | 212.7 a ± 14.2 | 203.7 a ± 19.3 | 22.5 a ± 1.6 | 1686.2 a ± 317 | 48.1 a ± 3.3 | 115.4 a ± 1.7 |
| T9 | 3000 | 220.8 a ± 21.0 | 136.7 a ± 1.7 | 9.6 a ± 1.5 | 1938.6 a ± 193 | 34.5 e ± 1.8 | 95.5 a ± 4.7 | 281.0 a ± 13.2 | 215.0 a ± 18.3 | 22.3 a ± 0.3 | 1916.5 a ± 276 | 47.1 a ± 2.6 | 119.8 a ± 8.9 |

[(1)] Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$)

± Values indicate standard deviation from the mean

**Table 3.5** Heavy metal content in must of Cabernet Sauvignon/99R grapevines irrigated using augmented winery wastewater during the 2010/11 and 2011/12 seasons (each value represents the average of triplicate treatments).

| Treatment no. | Target COD (mg/L) | 2010/11 | | | | |
|---|---|---|---|---|---|---|
| | | Cr (mg/L) | Cd (mg/L) | As (mg/L) | Pb (mg/L) | Hg (mg/L) |
| T1 | Raw water | 0.24 a[(1)] ± 0.06 | nd | nd | nd | nd |
| T2 | 100 | 0.33 a ± 0.04 | nd | nd | nd | nd |
| T3 | 250 | 0.29 a ± 0.08 | nd | nd | nd | nd |
| T4 | 500 | 0.43 a ± 0.10 | nd | nd | nd | nd |
| T5 | 1000 | 0.26 a ± 0.01 | nd | nd | nd | nd |
| T6 | 1500 | 0.34 a ± 0.10 | nd | nd | nd | nd |
| T7 | 2000 | 0.29 a ± 0.06 | 0.01 | nd | nd | nd |
| T8 | 2500 | 0.46 a ± 0.02 | nd | nd | nd | nd |
| T9 | 3000 | 0.40 a ± 0.10 | nd | nd | nd | nd |

[(1)] Values designated by the same letters within a column do not differ significantly (p≤0.05)

nd - Not detected

± Values indicate standard deviation from the mean

3.3.4.2.2 Total soluble solids, total titratable acidity and pH

Results of TSS, TTA and pH for the different treatments are presented in table 3.6. Irrigation using augmented winery wastewater did not have any effect on sugar accumulation during either of the two seasons when compared to the raw water irrigation control (Figs. 3.13 & 3.14).

Similarly, the evolution of TTA was not affected throughout the season (Figs. 3.15 & 3.16). Although the reduction in berry TTA for T7 was initially slower in the 2010/11 season, the titratable acidity concentration was similar by 16 February.

Juice pH was not affected by the level of wastewater augmentation at any stage of berry development up to harvest in the 2010/11 season (Figs. 3.17 & 3.18). In the 2011/12 season, juice pH for T9 was consistently higher than that of the other treatments, including the control. Furthermore, a clear tendency to higher juice pH at harvest was observed with an increase in level of COD of augmented winery wastewater. Still, only T9 had a significantly higher pH than the control treatment. The elevated pH with an increase in level of COD may be linked to a corresponding increase in Na and K applied through irrigation (Table 3.1). Where grapevines and other crops had been irrigated with Na- and K-rich water in the past, it had often led to increases in levels of these ions in plant tissue, resulting in increased juice pH (Somers, 1975; McCarthy, 1981; Neilsen *et al.,* 1989a; Mpelasoka *et al.,* 2003; Stevens *et al.,* 2011). However,

juice Na and K concentrations were similar for all treatments in the 2011/12 season, meaning that juice pH was increased without having elevated Na and/or K levels.

A higher crop load and possibly cooler climatic conditions in the 2011/12 season resulted in delayed berry ripening, compared to the 2010/11 season, due to longer hang time to reach the same sugar concentration. The differences in climatic conditions and vineyard practises between the two seasons may have affected the physiological functioning of the whole grapevine, leading to differences in absorption, ratios and impact of the minerals in vegetative and reproductive organs. The results nevertheless show that irrigation with winery wastewater may have affected physiological functioning of the grapevine due to increased Na and K uptake, resulting in increased must pH. Grapevine functioning with regards to sugar accumulation and organic acid degradation was, however, not influenced.

**Table 3.6** Chemical composition of grape must of Cabernet Sauvignon/99R grapevines irrigated using augmented winery wastewater during the 2010/11 and 2011/12 seasons (each value represents the average of triplicate treatments).

| Treatment no. | Target COD (mg/L) | 2010/11 | | | 2011/12 | | |
|---|---|---|---|---|---|---|---|
| | | Total soluble solids (°B) | pH | Total titratable acidity (g/L) | Total soluble solids (°B) | pH | Total titratable acidity (g/L) |
| T1 | Raw water | 23.6 a[(1)] ± 1.0 | 3.49 a ± 0.04 | 5.33 a ± 0.6 | 23.17 a ± 1.1 | 3.68 bc ± 0.12 | 5.38 a ± 0.4 |
| T2 | 100 | 23.5 a ± 0.7 | 3.47 a ± 0.05 | 5.45 a ± 0.7 | 22.50 a ± 0.2 | 3.67 bc ± 0.08 | 5.43 a ± 0.5 |
| T3 | 250 | 23.6 a ± 0.6 | 3.45 a ± 0.04 | 5.47 a ± 0.6 | 22.93 a ± 0.5 | 3.62 c ± 0.04 | 4.90 a ± 0.6 |
| T4 | 500 | 24.3 a ± 0.3 | 3.52 a ± 0.06 | 5.20 a ± 0.3 | 23.00 a ± 0.5 | 3.70 bc ± 0.04 | 4.98 a ± 0.4 |
| T5 | 1000 | 24.1 a ± 0.3 | 3.49 a ± 0.06 | 5.17 a ± 0.2 | 24.20 a ± 0.4 | 3.76 ab ± 0.03 | 4.17 a ± 0.6 |
| T6 | 1500 | 23.4 a ± 0.2 | 3.47 a ± 0.02 | 5.47 a ± 0.5 | 22.90 a ± 0.5 | 3.75 ab ± 0.02 | 4.75 a ± 0.4 |
| T7 | 2000 | 23.9 a ± 0.4 | 3.53 a ± 0.04 | 5.37 a ± 0.3 | 22.57 a ± 0.7 | 3.76 ab ± 0.05 | 6.03 a ± 0.4 |
| T8 | 2500 | 23.6 a ± 0.4 | 3.52 a ± 0.08 | 5.47 a ± 0.1 | 22.73 a ± 0.5 | 3.77 ab ± 0.11 | 4.77 a ± 0.3 |
| T9 | 3000 | 24.7 a ± 0.3 | 3.57 a ± 0.04 | 4.82 a ± 0.4 | 24.10 a ± 0.5 | 3.85 a ± 0.08 | 4.97 a ± 0.3 |

[(1)] Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$)

± Values indicate standard deviation from the mean

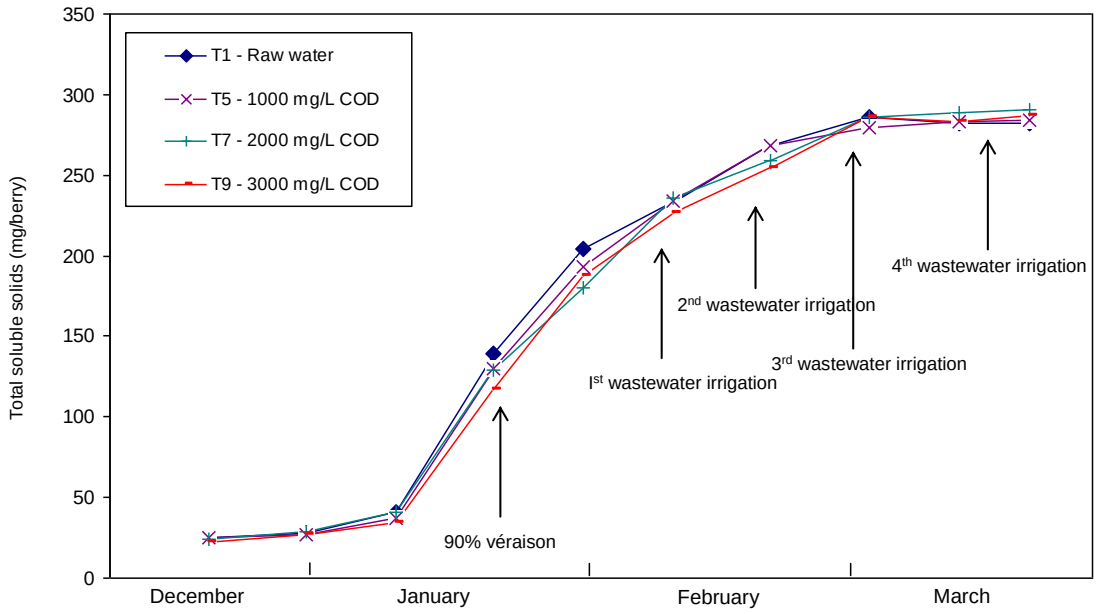


**Fig 3.13** The effect of irrigation using augmented winery wastewater on total soluble solids in berries of Cabernet Sauvignon/99R grapevines during ripening in the 2010/11 season. Indicated values represent the average of triplicate treatments (standard deviation not shown).

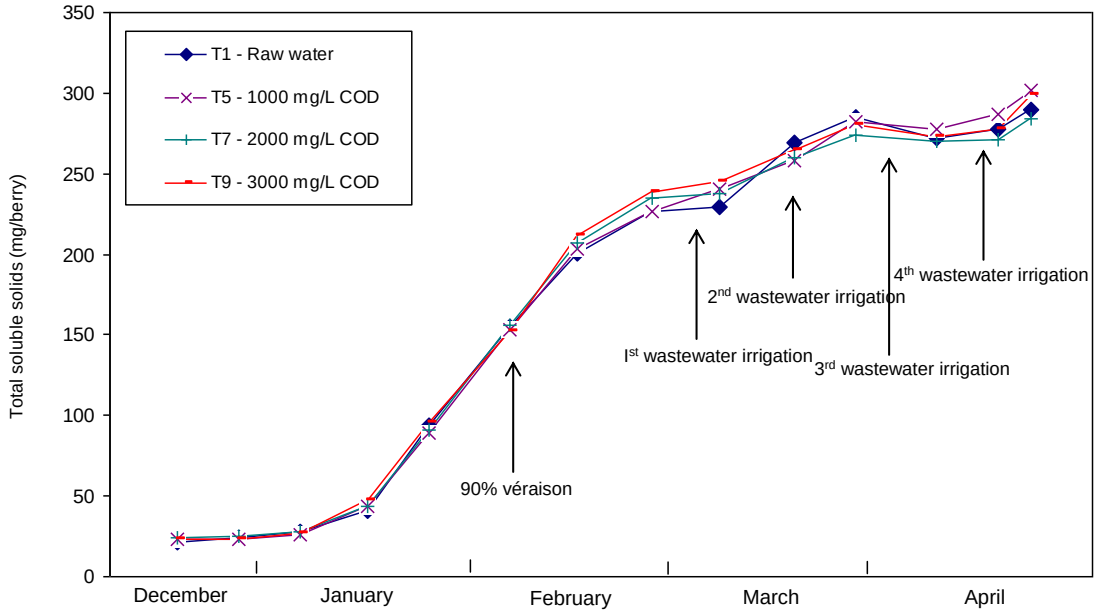


**Fig 3.14** The effect of irrigation using augmented winery wastewater on total soluble solids in berries of Cabernet Sauvignon/99R grapevines during ripening in the 2011/12 season. Indicated values represent the average of triplicate treatments (standard deviation not shown).

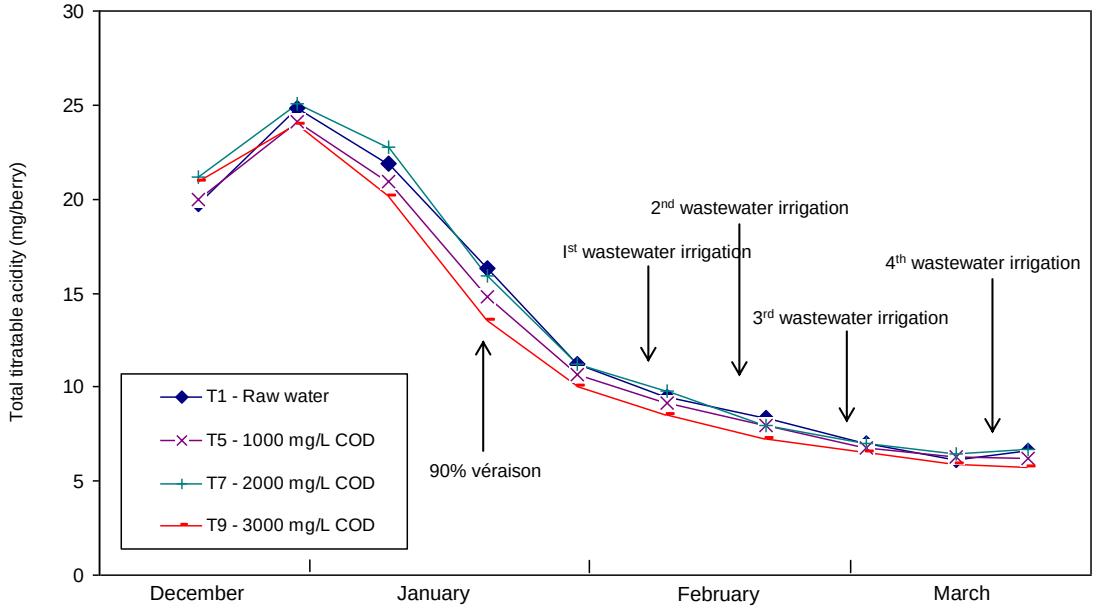


**Fig 3.15** The effect of irrigation using augmented winery wastewater on total titratable acidity in berries of Cabernet Sauvignon/99R grapevines during ripening in the 2010/11 season. Indicated values represent the average of triplicate treatments (standard deviation not shown).

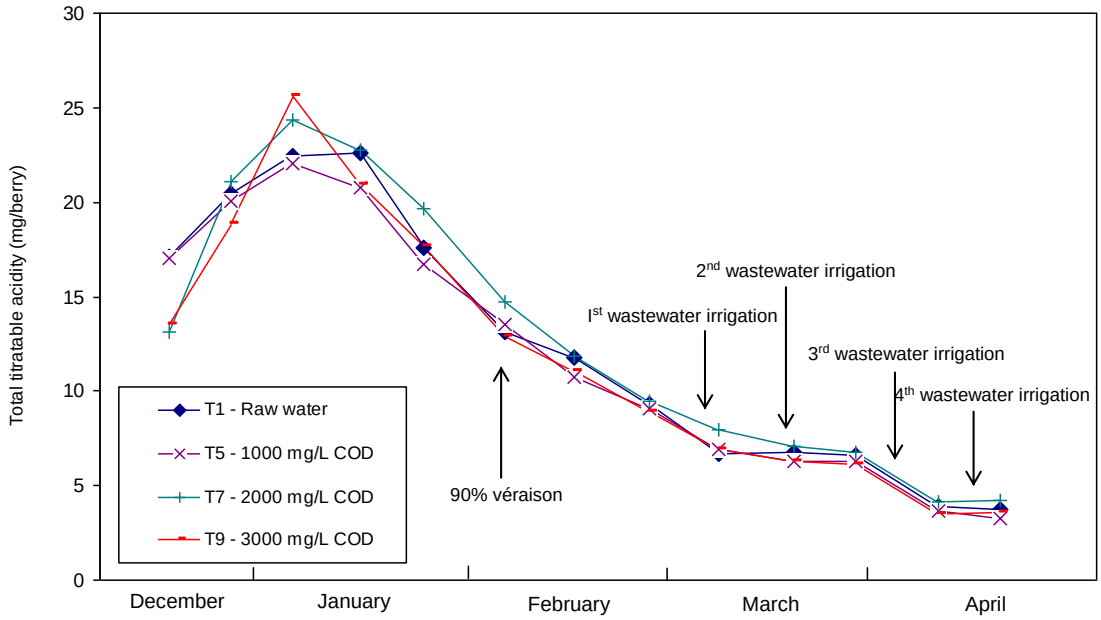


**Fig 3.16** The effect of irrigation using augmented winery wastewater on total titratable acidity in berries of Cabernet Sauvignon/99R grapevines during ripening in the 2011/12 season. Indicated values represent the average of triplicate treatments (standard deviation not shown).

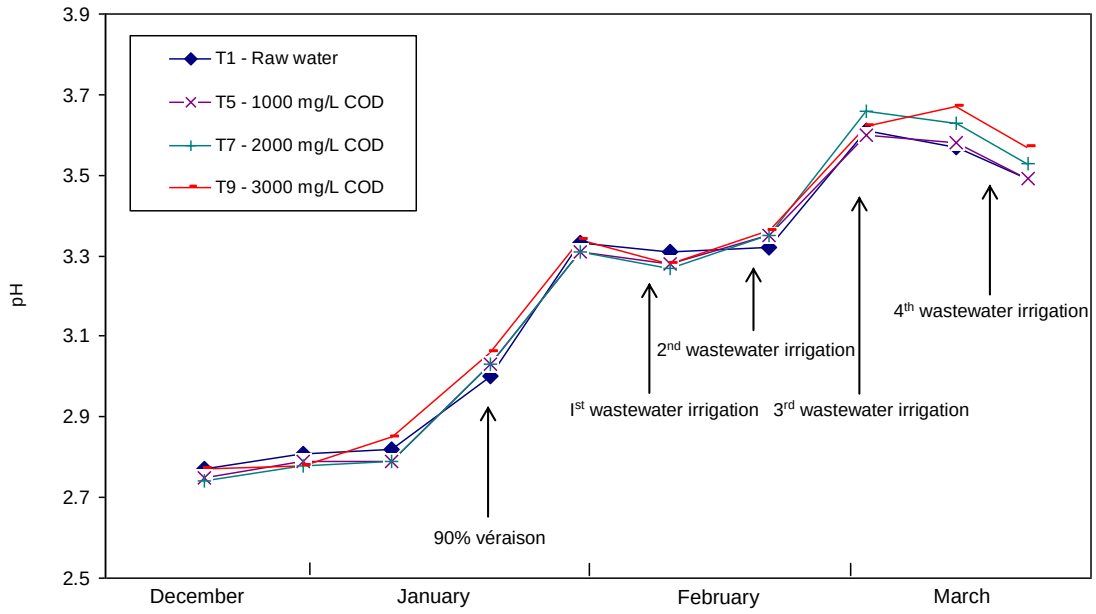


**Fig 3.17** The effect of irrigation using augmented winery wastewater on juice pH of Cabernet Sauvignon/99R grapevines during ripening in the 2010/11 season. Indicated values represent the average of triplicate treatments (standard deviation not shown).

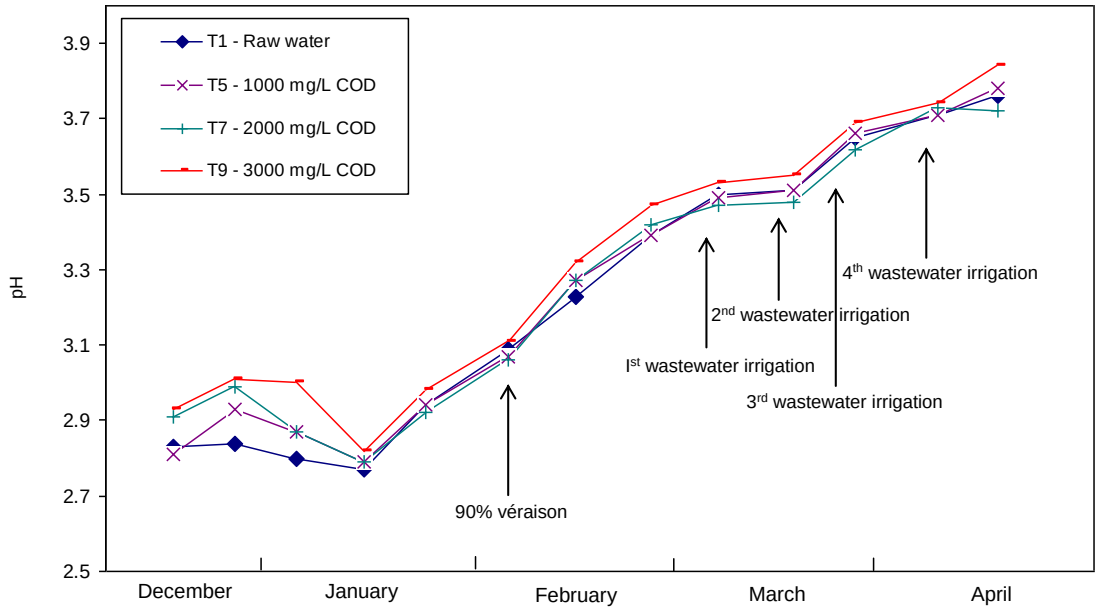


**Fig 3.18** The effect of irrigation using augmented winery wastewater on juice pH of Cabernet Sauvignon/99R grapevines during ripening in the 2011/12 season. Indicated values represent the average of triplicate treatments (standard deviation not shown).

3.3.4.2.3 Tartaric acid and malic acid

The two dominant and most important organic acids in grape berries are tartaric acid and malic acid, comprising 72% to 98% of total berry organic acid content (Kliewer, 1966; Coombe, 1992). Berry ripening is coupled with a reduction in berry malic acid concentration due to acid degradation as well as dilution. On the other hand, a decline in tartaric acid concentration only occurs because of dilution (Esteban *et al*., 1999). In this study, tartaric acid concentration exceeded malic acid concentration at all three points of sampling (Figs. 3.19 & 3.20).

Esteban *et al.* (1999) reported tartaric acid to dominate the acid composition towards the later stages of berry development. Berry malic acid experienced a rapid decline in the 10 day interval between the first two sampling dates after which it decreased at a very slow rate until harvest. Tartaric acid concentration decreased continuously throughout berry ripening, even when berries had reached its maximum volume. Furthermore, contrary to literature, the tartaric acid decline was more rapid than that of malic acid degradation during the measured time intervals. Due to the stability of tartaric acid it cannot be degraded, but only be diluted. Therefore, the continuous decline in measured tartaric acid concentration may have been a result of the method used for tartaric acid quantification. Only tartaric acid present in its free form was measured by the tartaric acid enzymatic kit. Thus, any tartaric acid that had been converted into its tartrate salt would not be measured by this method. The continued decrease in tartaric acid concentration during ripening could therefore be explained by its conversion from free tartaric acid to tartrate salts.

Even though an increase in the amount of Na and K applied to the soil was observed with an increase in the COD level of augmented winery wastewater (Table 3.1), these two major organic acids were not affected by any of the selected wastewater irrigation treatments (Figs. 3.19 & 3.20). Although higher malic acid concentrations were observed for T7 and T9 on the first sampling date, values were similar at later stages during the season. Therefore, the observation made by various authors stating that excessive Na and K fertilization could cause increased salt formation from malic and tartaric acid, with a subsequent decrease in these acids and TTA, was not observed in this study (Iland and Coombe, 1988; Mpelsoka *et al.,* 2003). The reason for this was probably because K and Na were not increased in the grape juice (Table 3.4).

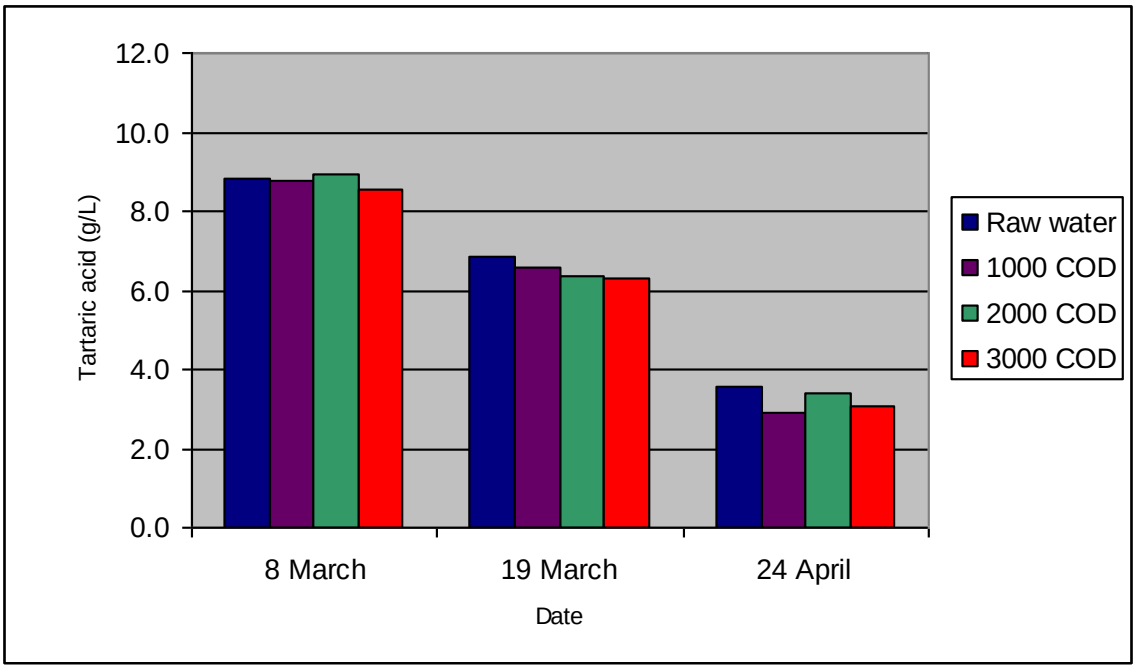


**Fig 3.19** The effect of irrigation using augmented winery wastewater on berry tartaric acid concentration of Cabernet Sauvignon/99R grapevines during ripening in the 201/12 season. Indicated values represent the average of triplicate treatments (standard deviation not shown).

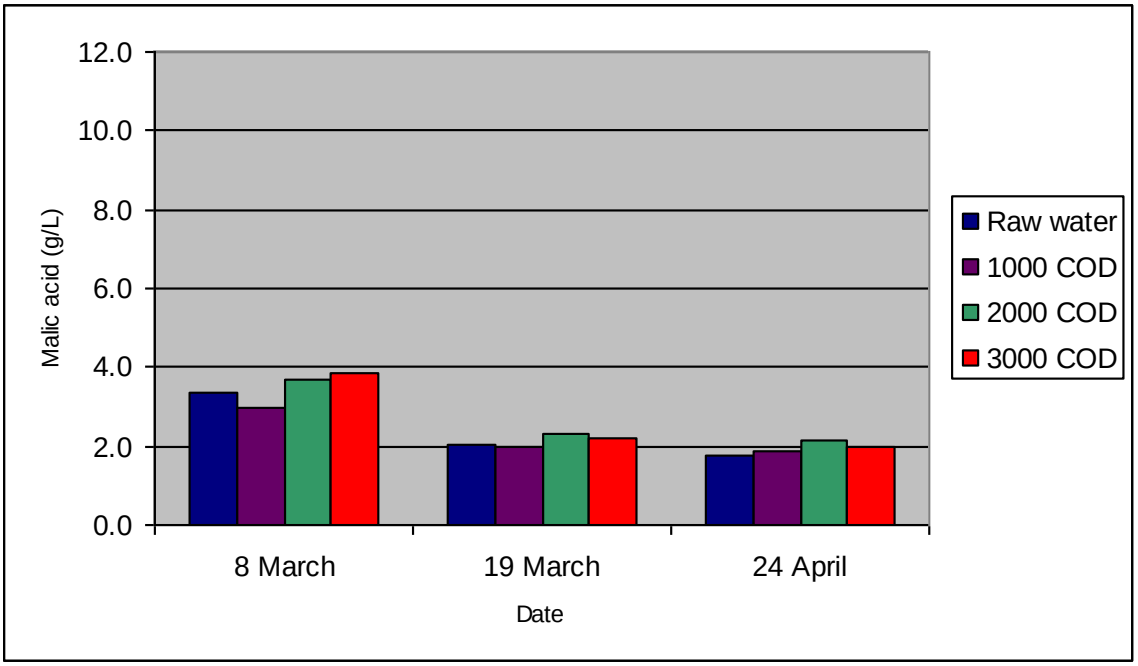


**Fig 3.20** The effect of irrigation using augmented winery wastewater on berry malic acid concentration of Cabernet Sauvignon/99R grapevines during ripening in the 201/12 season. Indicated values represent the average of triplicate treatments (standard deviation not shown).

3.3.4.3 Berry skin characteristics

Berry skin wet mass and dry mass were similar for all wastewater irrigation treatments and the control in both seasons (Table 3.7), thus indicating that wastewater irrigation had no effect on berry skin size and thickness. Furthermore, regardless of level of COD in the augmented winery wastewater, no differences in anthocyanin content of berries at $A_{420}$ or $A_{520}$ were found at ripeness in either of the two seasons (Table 3.7). Therefore, grape berries contained similar concentrations of brown ($A_{420}$) and red ($A_{520}$) colour pigments at harvest. The total amount of phenolic compounds contained in berry skins was also similar for all treatments in both seasons (Table 3.7). Even though total phenol concentrations were not affected, individual phenolic compounds may have been affected. Analysis of individual phenolic compounds was however beyond the scope of this study. These similarities indicate that grapevine microclimate as well as grapevine functioning with regards to colour pigment and phenolic compound accumulation was not affected by the various wastewater irrigation treatments during ripening.

### 3.3.5 Yield components at harvest

Reproductive parameters were determined at harvest in 2011 and 2012. Berry mass and volume at harvest were discussed earlier. The number of bunches per grapevine was not affected by any of the wastewater irrigation treatments, with the exception of T7 having less bunches in the 2010/11 season (Table 3.3). The lower number of bunches per grapevine for T7 was coincidental and could not be attributed to a treatment effect. Bunch mass for all treatments was similar in both seasons (Table 3.3). Therefore, the previously observed increase in fruit size and fruit per tree for tomatoes, apples and Riesling grapevines due to irrigation using treated wastewater (Neilsen *et al*., 1989a, Neilsen *et al*., 1989b; Al-Lahham *et al.,* 2003), was not observed on grapevine bunch size and numbers in this study. These increases also resulted in higher yields for these crops. As the similarities in the number of bunches, bunch size and berry size imply, the various wastewater irrigation treatments did not have any effect on grapevine yield when compared to the raw water control in either season (Table 3.3). Grapevines from T7 and T9 produced lower yields than grapevines from some of the other treatments in the 2010/11 season, with T9 having smaller berries and T7 having fewer bunches per vine. There were no apparent differences in yield between any of the irrigation treatments in the 2011/12 season which suggests that the differences within the 2010/11 season were coincidental and/or due to variation in vigour. A drastic increase in yield was observed from the 2010/11 to the 2011/12 season. The increase in yield could be attributed to a combination of factors of which an increase in number of bunches per grapevine and bunch mass (therefore fertility and berry set) were most likely. A better grapevine structure and more shoots per vine in the 2011/12 season along with better climatic conditions during the previous season before flowering and in the 2011/12 season during initial growth and berry set may also have played a role.

**Table 3.7** Berry skins characteristics of grapes from Cabernet Sauvignon/99R grapevines irrigated using augmented winery wastewater during the 2010/11 and 2011/12 seasons (each value represents the average of triplicate treatments).

| Treatment no. | Target COD (mg/L) | 2011 | | | | | 2012 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Wet mass (g/berry) | Dry mass (g/berry) | Colour ($A_{420\ nm}$) | Colour ($A_{520\ nm}$) | Total phenols ($A_{280\ nm}$) | Wet mass (g/berry) | Dry mass (g/berry) | Colour ($A_{420\ nm}$) | Colour ($A_{520\ nm}$) | Total phenols ($A_{280\ nm}$) |
| T1 | Raw water | 0.420 a[(1)] ± 0.03 | 0.126 a ± 0.00 | 0.14 a* ± 0.02 | 0.53 a* ± 0.11 | 0.65 a* ± 0.14 | 0.409 a ± 0.05 | 0.091 a ± 0.02 | 0.19 a* ± 0.04 | 0.69 a* ± 0.15 | 0.89 a* ± 0.17 |
| T2 | 100 | 0.410 a ± 0.03 | 0.136 a ± 0.03 | 0.16 a ± 0.01 | 0.57 a ± 0.07 | 0.71 a ± 0.11 | 0.446 a ± 0.03 | 0.096 a ± 0.01 | 0.17 a ± 0.01 | 0.62 a ± 0.06 | 0.79 a ± 0.03 |
| T3 | 250 | 0.436 a ± 0.02 | 0.126 a ± 0.00 | 0.17 a ± 0.01 | 0.61 a ± 0.07 | 0.74 a ± 0.08 | 0.457 a ± 0.03 | 0.093 a ± 0.00 | 0.19 a ± 0.04 | 0.72 a ± 0.19 | 0.95 a ± 0.20 |
| T4 | 500 | 0.364 a ± 0.08 | 0.111 a ± 0.02 | 0.15 a ± 0.03 | 0.59 a ± 0.14 | 0.72 a ± 0.15 | 0.409 a ± 0.03 | 0.084 a ± 0.01 | 0.19 a ± 0.02 | 0.68 a ± 0.15 | 0.93 a ± 0.18 |
| T5 | 1000 | 0.392 a ± 0.02 | 0.116 a ± 0.00 | 0.14 a ± 0.02 | 0.56 a ± 0.07 | 0.66 a ± 0.04 | 0.424 a ± 0.04 | 0.093 a ± 0.01 | 0.21 a ± 0.01 | 0.79 a ± 0.11 | 1.02 a ± 0.15 |
| T6 | 1500 | 0.358 a ± 0.05 | 0.106 a ± 0.02 | 0.16 a ± 0.01 | 0.59 a ± 0.06 | 0.72 a ± 0.11 | 0.437 a ± 0.07 | 0.093 a ± 0.02 | 0.20 a ± 0.01 | 0.71 a ± 0.11 | 0.93 a ± 0.12 |
| T7 | 2000 | 0.367 a ± 0.01 | 0.110 a ± 0.01 | 0.16 a ± 0.01 | 0.59 a ± 0.08 | 0.71 a ± 0.13 | 0.385 a ± 0.02 | 0.077 a ± 0.00 | 0.24 a ± 0.02 | 0.88 a ± 0.08 | 1.26 a ± 0.12 |
| T8 | 2500 | 0.396 a ± 0.02 | 0.118 a ± 0.01 | 0.15 a ± 0.02 | 0.59 a ± 0.08 | 0.70 a ± 0.10 | 0.379 a ± 0.02 | 0.079 a ± 0.00 | 0.22 a ± 0.01 | 0.81 a ± 0.05 | 1.12 a ± 0.13 |
| T9 | 3000 | 0.410 a ± 0.00 | 0.142 a ± 0.03 | 0.16 a ± 0.01 | 0.64 a ± 0.05 | 0.74 a ± 0.05 | 0.451 a ± 0.02 | 0.097 a ± 0.00 | 0.20 a ± 0.01 | 0.72 a ± 0.02 | 0.94 a ± 0.07 |

[(1)] Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$)

*Values refer to absorbance units

± Values indicate standard deviation from the mean

There is a distinct relationship between crop load and grapevine vegetative growth. A decrease in grapevine weight, and thus vegetative growth, is coupled with an increase in crop (Winkler, 1974). Grapevine shoot tips and bunches are both sinks and therefore compete for photosynthetic products, mainly produced by the leaves (sources). If photosynthetic products are excessively translocated to shoot tips, at the cost of bunches, it may lead to a decline in fruit size and yield. Therefore, the higher crop load observed in the 2011/12 season in this study may have resulted in the decline in vegetative growth per shoot and better yield:growth balances.

## 3.4 CONCLUSIONS

Under the given conditions, the various wastewater irrigation treatments did not have any effect on plant water status and, therefore, grapevine vegetative growth, reproductive growth and yield. This indicates that the osmotic potential of the soil solution was not decreased to an extent which impeded grapevine water uptake. As grapevine vegetative growth was not affected by irrigation using augmented winery wastewater, which indicates that the grapevine microclimate was not effected either.

Accumulation of soluble solids and evolution in acidity is not affected by wastewater irrigation at any stage during the growing seasons. For the most part, grapevine functioning is not affected by irrigation using augmented winery wastewater. Winery wastewater irrigation may, however, induce changes in grapevine functioning due to increased uptake of Na and K and cause an increase in juice pH throughout berry development up to harvest. With the exception of a possible increase in must Na and decrease in must Ca, none of the measured elements, cations and heavy metals in the must were affected by irrigation with winery wastewater within the applied ranges of this study. Juice Na increased, while juice Ca decreased with an increase in the level of COD of augmented winery wastewater. Must N, P, cation and heavy metal content for wastewater irrigated grapevines were all within the norms for wine grapes. Thus, the increase in amounts of P and cations applied up to harvest *via* wastewater irrigation does not increase the degree to which these elements and cations are absorbed, or they are not released into must during normal preparations for must analysis.

Berry skin mass, anthocyanins and phenol concentration are probably not affected by irrigation using augmented winery wastewater. The ability of grape berries to synthesise and accumulate anthocyanins and phenolic compounds are, therefore, not affected when grapevines are irrigated with augmented winery wastewater.

Conclusions drawn from this chapter suggest that wastewater irrigation within the applied ranges may be suitable for vineyard irrigation without having any major negative impacts on grapevine performance and juice composition. Although irrigation using augmented winery wastewater did not alter any of the measured vegetative or reproductive growth parameters in this two year trial, long term effects may be different. Furthermore, using a different rootstock cultivar may have yielded different results. The increased amounts of certain elements, such as Na, applied through wastewater irrigation may alter soil physical and chemical composition and, therefore, grapevine growth. Furthermore, if these elements are absorbed into grapevine berries, they may affect the functioning and composition of the berries. The increase in juice pH, observed in the 2011/12 season, may also be more severe if soil Na and K are considerably higher.

This study serves as a baseline for future investigations into the effects of irrigation using winery wastewater on grapevine growth, juice and wine composition and wine quality in the South African wine industry. Future studies should incorporate the effects that different soils and winery wastewater from different localities might have. Furthermore, as juice and must acidity and pH was affected the most in this study, future work should focus on this and include measurements of a wider range.

## 3.5 LITERATURE CITED


Al-Lahham, O., El Assi, N. & Fayyad, M., 2003. Impact of treated wastewater irrigation on quality attributes and contamination of tomato fruit. Agr. Water Manage. 61, 51-62.

Anderson, K.C., Findlay, C., Fuentes, S. & Tyerman, S., 2008. Viticulture, wine and climate change. Garnaut Climate Change Review, Adelaide, Australia. pp. 1-22. (www.garnautreview.org.au).

ANZECC, 2000. Australian and New Zealand guidelines for fresh and marine water quality: Volume 3 - primary industries - rationale and background information. Chapter 9. (http://www.environment.gov.au/water/publications/quality/pubs/nwqms-guidelines-4-vol3.pdf).

Ayers, R.S. & Westcott, D.W., 1985. Water quality for agriculture, FAO Irrigation and Drainage Paper No. 29, FAO, Rome. pp. 1-96. (http://www.calwater.ca.gov/Admin_Record/C-110101.pdf).

Bernstein, L., 1975. Effects of salinity and sodicity on plant growth. Annu. Rev. Phytopathol. 13, 295-312.

Bolan, N.S., Horne, D.J. & Currie, L.D., 2004. Growth and chemical composition of legume-based pasture irrigated with farm effluent. N.Z. J. Agr. Res. 47, 85-93.

Boulton, R., 1980. The general relationship between potassium, sodium and pH in grape juice and wine. Am. J. Enol. Vitic. 31, 182-186.

Clesceri, L.S., Greenberg, A.E. & Eaton, A.D., 1998 (20th ed). Standard methods for the examination of water and waste water. Am. J. Public Health 4, 117-122.

Coetzee, G., Malandra, L., Wolfaardt, G.M. & Viljoen-Bloom, M., 2004. Dynamics of a microbial biofilm in a rotating biological contactor for the treatment of winery effluent. Water SA 30, 407-412.

Conradie, W.J., 2001. Timing of nitrogen fertilisation and the effect of poultry manure on the performance of grapevines on sandy soil. II. Leaf analysis, juice analysis and wine quality. S. Afr. J. Enol. Vitic. 22, 60-68.

Coombe, B.G., 1992. Research on development and ripening of the grape berry. Am. J. Enol. Vitic. 43, 101-110.

Department of Water Affairs and Forestry (DWAF), 1996. South African water quality guidelines. Vol. 4, Agricultural use: irrigation. CSIR Environmental Services. Department of Water Affairs and Forestry, Pretoria

Department of Water Affairs and Forestry, 2004. Revision of general authorisation in terms of Section 39 of the National Water Act, 1998 (Act 36 of 1998). Section 21, Government Notice 1091, Government Gazette 26187, 13, 1-33. August 1998, Department of Water Affairs and Forestry, Pretoria, South Africa.

Esteban, A., Villanueva, J. & Lissarrague, J.R., 1999. Effect of irrigation on changes in berry composition of Tempranillo during maturation. Sugars, organic acids, and mineral elements. Am. J. Enol. Vitic. 50, 418-434.

Garcia, M., Daverede, C., Gallego, P. & Toumi, M., 1999. Effect of various potassium-calcium ratios on cation nutrition of grape grown hydroponically. J. Plant Nutr. 22, 417-425.

Grattan, S.R. & Grieve, C.M., 1998. Salinity-mineral nutrient relations in horticultural crops. Sci. Hortic. 78, 127-157.

Haight, K.G. & Gump, B.H., 1995. Red and white grape juice concentrate component ranges. J. Food Compos. Anal. 8, 71-77.

Hamilton, A.J., Boland, A.M., Stevens, D., Kelly, J., Radcliffe, J., Ziehrl, A., Dillon, P. & Paulin, B., 2005. Position of the Australian horticultural industry with respect to the use of reclaimed water. Agr. Water Manage. 71, 181-209.

Hunter, J.J., De Villiers, O.T. & Watts, J.E., 1991. The effect of partial defoliation on quality characteristics of *Vitis vinifera L.* cv. Cabernet Sauvignon grapes. II. Skin color, skin sugar, and wine quality. Am. J. Enol. Vitic. 42, 13-18.

Jourjon, F., Khaldi, S., Reveillere, M., Thibault, C., Poulard, A., Chretien, P. & Bednar, J., 2005. Microbiological characterization of winery effluent: an inventory of the sites for different treatment systems. Water Sci. Technol. 51, 19-26.

Iland, P.G. & Coombe, B.G., 1988. Malate, tartrate, potassium and sodium in flesh and skin of Shiraz grapes during ripening: concentration and compartementation. Am. J. Enol. Vitic. 39, 71-76.

Iland, P., Ewart, A., Sitters, J., Markides, A. & Bruer, N., 2000. Techniques for chemical analysis and quality monitoring during winemaking. Patrick Iland Wine Promotions Pty Ltd. Campbelltown, Australia.

International Organisation of Vine and Wine (OIV). Compendium of international methods of analysis – OIV. The level of sodium and chloride in wine. (www.oiv.int/oiv/cms/index?lang=en).

Kinge, C.W. & Mbewe, M., 2012. Bacterial contamination levels in river catchments of the North West Province, South Africa: public health implications. Afr. J. Microbiol. Res. 6, 1370-1375.
Kliewer, W.M., 1966. Sugars and organic acids of Vitis vinifera. Plant Physiol. 41, 923-931.

Kumar, A., Arienzo, M., Quayle, W., Christen, E., Grocke, S., Fattore, A., Doan, H., Gonzago, D., Zandonna, R., Bartrop, K., Smith, L., Correl, R. & Kookana, R., 2009. Developing a systematic approach to winery wastewater management. CSIRO Land and Water Science Report. pp. 1-131.

Lategan, E.L., 2011. Determining of optimum irrigation schedules for drip irrigated Shiraz vineyards in the Breede River Valley. MSc thesis, Stellenbosch University, Private Bag X1, 7602 Matieland (Stellenbosch), South Africa.

Laurenson, S., Bolan, N., Smith, E. & McCarthy, M., 2010. Winery wastewater irrigation: effects of sodium and potassium on soil structure. CRC CARE Technical Report 19, 1-25. (http://www.crccare.com/publications/downloads/CRC-CARE-Tech-Report-19.pdf).

Malandra, L., Wolfaardt, G., Zietsman, A., & Viljoen-Bloom, M., 2003. Microbiology of a biological contactor for winery wastewater treatment. Water res. 37, 4125-4134.

Marschner, H., 1986. Mineral nutrition of higher plants. Academic Press, London.

Mattick, L.R., Shaulis, N.J. & Moyer, J.C., 1972. The effect of potassium fertilization on the acid content of 'Concord' grape juice. Am. J. Enol. Vitic. 23, 26-30.

McCarthy, M.G., 1981. Irrigation of grapevines with sewage effluent. I. Effects on yield and petiole composition. Am. J. Enol. Vitic. 32, 189-196.

McCarthy, M.G., Cirami, R.M. & McCloud, P., 1983. Vine and fruit responses to supplementary irrigation and canopy management. S. Afr. J. Enol. Vitic. 4, 67-76.

McCarthy, M.G., Jones, L.D. & Due, G., 1992. Irrigation - principles and practices. In: Coombe, B. G. & Dry, P. R. (eds). Viticulture Volume 2 - Practices. Winetitles, Adelaide. pp. 104-128.

Morris, J., Sims, C. & Cawthon, D., 1983. Effects of excessive potassium levels on pH, acidity and color of fresh and stored grape juice. Am. J. Enol. Vitic. 34, 35-39.

Mosse, K.P.M., Patti, A.F., Christen, E.W. & Cavagnaro, T.R., 2010. Winery wastewater inhibits seed germination and vegetative growth of common crop species. J. Hazard. Mater. 180, 63-70.

Mpelasoka, B.S., Schachtman, D.R., Treeby, M.T. & Thomas, M.R., 2003. A review of potassium nutrition in grapevines with special emphasis on berry accumulation. Aust. J. Grape Wine Res. 9, 154-168.

Mulidzi, A.R., Wooldridge, J., Laker, M.C. & Van Schoor, L., 2009. Composition of effluents from wineries in the Western and Northern Cape Provinces I. Seasonal variations and differences between wineries. Wineland, January. pp. 88-91.

Munns, R. & Termaat, A., 1986. Whole-plant responses to salinity. Aust. J. Plant Physiol. 13, 143-160.

Myburgh, P.A., 2006. Juice and wine quality responses of *Vitis vinifera* L. *cvs. S*auvignon blanc and Chenin blanc to timing of irrigation during berry ripening in the coastal region of South Africa. S. Afr. J. Enol. Vitic. 27, 1-7.

Myburgh, P.A., 2011. Response of *Vitis vinifera* L. cv. Merlot to low frequency irrigation and partial root zone drying in the Western Cape coastal region – Part II. Vegetative growth, yield and quality. S. Afr. J. Enol. Vitic. 32, 104-116.

Myburgh, P.A., 2012. Guidelines for irrigation of vineyards with saline water. Submitted for publication.

Neilsen, G., Stevenson, D. & Fitzpatrick, J., 1989a. The effect of municipal wastewater irrigation and rate of N fertilization on petiole composition, yield and quality of Okanagan Riesling grapes. Can. J. Plant Sci. 69, 1285-1294.

Neilsen, G., Stevenson, D., Fitzpatrick, J. & Brownlee, C., 1989b. Nutrition and yield of young apple trees irrigated with municipal waste water. J. Am. Soc. Hortic. Sci. 114, 377-383.

Ough, C.S. & Kriel, A., 1985. Ammonia concentrations of must of different grape cultivars and vineyards in the Stellenbosch area. S. Afr. J. Enol. Vitic. 6, 7-11.

Paulse, A.N., Jackson, V.A. & Khan, W., 2009. Comparison of microbial contamination at various sites along the Plankenburg- and Diep Rivers, Western Cape, South Africa. Water SA 35, 469-477.

Prior, L.D., Grieve, A.M. & Cullis, B.R., 1992. Sodium chloride and soil texture interactions in irrigated field grown Sultana grapevines. II. Plant mineral content, growth and physiology. Aust. J. Agr. Res. 43, 1067-1083.

Ryder, R.A., 1995. Aerobic pond treatment of winery wastewater for vineyard irrigation by drip and spray system in California. Rev. Fr. Oenol. 152, 22-24.

Saayman, D., 1981. Wingerdvoeding. In: Burger, J. & Deist, J. (eds). Wingerdbou in Suid-Afrika. ARC Infruitec-Nietvoorbij, Stellenbosch, South Africa. pp. 343-371.

Samra, J.S., 1985. Sodicity tolerance of grapes with reference to the uptake of nutrients. Indian J. Hortic. 42, 12-17.

Scherer, T.F., Seelig, B. & Franzen, D., 1996. Soil, water and plant characteristics important to irrigation. Extension Bulletin EB-66, North Decota State University Extension Service, Fargo, North Dacota. pp. 15.

Sheridan, C.M., Glasser, D., Hildebrandt, D., Petersen, J. & Rohwers, J., 2011. An annual and seasonal characterisation of winery effluent in South Africa. S. Afr. J. Enol. Vitic. 32, 1-8.

Somers, T.C., 1975. In search of quality for red wines. Food Technol. Aust. 27, 49-56.

South Australian Environmental Protection Authority, 2004. Guidelines for wineries and distilleries. January. pp. 1-20. (www.apal.com.au/site/DefaultSite/filesystem/.../EPAguidelines.pdf).

Stevens, R.M., Harvey, G., Partington, D.L. & Coombe, B.G., 1999. Irrigation of grapevines with saline water at different growth stages: effect on soil, vegetative growth and yield. Aust. J. Agric. Res. 50, 343-355.

Stevens, R.M., Harvey, G. & Partington, D.L., 2011. Irrigation of grapevines with saline water at different growth stages: effect on leaf, wood and juice composition. Aust. J. Grape Wine Res. 17, 239-248.

Van Schoor, L.H., 2005. Guidelines for management of wastewater and solid waste at existing wineries. Winetech, South Africa. (http://www.winetech.co.za/index.php).

Van Schoor, L.H. & Mulidzi, A.R., 2001. Ondersoek na die omgewingsimpak en gevolglike bestuur van uitvloeisel vanaf brandewyn en spiritus stokerye en wynkelders. Unpublished report, Winetech, Cape Town.

Van Zyl, J.L., 1981. Waterbehoefte en besproeiing. In: Burger, J. & Deist, J. (eds). Wingerdbou in Suid-Afrika. ARC Infruitec-Nietvoorbij, Stellenbosch, South Africa. pp. 234-282.

Walker, R.R., Torokfalvy, E., Steele Scott, N. & Kriedemann, P.E., 1981. An analysis of photosynthetic response to salt treatment in *Vitis vinifera*. Aust. J. Plant Physiol. 8, 359-374.

Walker, R.R., Blackmore, D.H., Clingeleffer, P.R. & Iacono, F., 1997. Effect of salinity and Ramsey rootstock on ion concentrations and carbon dioxide assimilation in leaves of drip irrigated, field-grown grapevines (*Vitis vinifera* L. cv. Sultana). Aust. J. Grape Wine Res. 3, 66-74.

Williams, L.E., Dokoozlian, N.K. & Wample, R., 1994. Grape. In: Schaffer, B. & Anderson, P.C. (eds). Handbook of Environmental Physiology of Fruit Crops, Vol. I. Temperate Crops. CRC Press, Orlando. pp. 83-133.

Winkler, A.J., Cook, J.A., Kliewer, W.M. & Lider, L.A., 1974 ($4^{th}$ ed). General viticulture. University of California Press, Berkeley.

# Chapter 4

## Research results

### The effect of irrigation using winery wastewater on juice and wine microbial flora, wine chemical composition and sensorial characteristics of Cabernet Sauvignon/99R in the Breede River Valley

# 4. RESEARCH RESULTS

## 4.1 INTRODUCTION

Climatic conditions such as temperature, radiation, humidity, rainfall, evaporation, wind and water availability are crucial factors in determining grapevine performance and wine style and quality (Van Leeuwen and Seguin, 2006). The availability of water is, arguably, one of the most important wine production and quality determining factors. A decrease in wine quality can be expected if water shortages arise and no irrigation is applied (Williams *et al.,* 1994). As a decrease in water availability is expected for South Africa in the near future (Department of Environmental Affairs and Tourism, 2004), finding additional sources of water for irrigation is becoming a necessity. If irrigation using augmented winery wastewater can develop into a sustainable practice in the wine industry, it may serve as one of these supplementary sources of irrigation water. However, irrigation water quality is also an important factor influencing grapevine growth and juice and wine composition and should be considered carefully before irrigation is applied.

The quality of winery wastewater varies considerably between wineries and at different periods during the year (Chapman, 1996; Van Schoor, 2005; Mulidzi *et al.,* 2009). Winery wastewater contains high COD concentrations and higher concentrations of certain elements, particularly sodium (Na) and potassium (K), than water generally used for crop irrigation (Mulidzi *et al.,* 2009; Sheridan *et al.,* 2011). When grapevines are irrigated with water that is rich in Na and/or K it may lead to increased levels of these ions in grapevine tissue and berries (McCarthy, 1981; Neilsen *et al.,* 1989; Stevens *et al.,* 2011).

Increased Na and K levels in grape berries can cause a decrease in available free malic and tartaric acid content, resulting in an increase in juice pH (Somers, 1975; Mpelasoka *et al.,* 2003; Stevens *et al.,* 2011). An increase in juice pH would lead to a subsequent increase in pH of the resulting wine. Increases in wine pH may be beneficial in certain cool winegrowing regions of the world. However, with its generally moderate to warm temperatures, a lower wine pH is preferred in South Africa. Wines with high pH values generally taste flat and result in red wines with an undesirable brownish hue (Gladstones 1992; Rühl 1989). Furthermore, as wine pH increase, the anthocyanin equilibrium shifts away from the red flavilium cation form, towards the colourless, yellow and blue forms, reducing wine red colour hue (Ribéreau-Gayon *et al.,* 2006).

The presence of Na in grapes that are used to make wine can increase the amount of undesirable phenolic compounds in wine, severely reducing wine quality (White, 2003). If the element applied through wastewater irrigation are absorbed by the grapevine and increased in

grapes, it may lead to an increase in these elements in wines made from these grapes. Rankine *et al.* (1971) and Donkin *et al.* (2010) reported that the white wine vinification process does not decrease juice Na concentration. Therefore, wines made from juice with a high Na concentration will contain at least the same concentration of Na. The longer periods of skin contact during red wine vinification may result in even higher concentrations of certain elements due to increased extraction from berry skins, resulting in an even larger decrease in wine quality. The legal limit for Na in South African wines is 100 mg/L (Department of Water Affairs and Forestry, 1996).

Winery wastewater has been known to cause soil salinity (Van Schoor, 2005). The large amounts of Na applied *via* winery wastewater irrigation may bind with chloride (Cl) present in the soil to form high sodium chloride (NaCl) concentrations. Sodium and Cl ions are then taken up by the grapevine *via* passive diffusion and subsequently enter the berries (Stevens and Walker, 2002; Stevens *et al.,* 2011). Furthermore, increases in the uptake of these ions lead to an increase in juice NaCl. Although the NaCl threshold of wine is unknown, high NaCl concentrations in wines result in wines with a flat, dull, salty, brackish and soapy character (Walker *et al.,* 2003). The presence of NaCl causes osmotic stress and ads to the hostile environment in which yeast and lactic acid bacteria (LAB) cells need to conduct alcoholic fermentation (AF) and malolactic fermentation (MLF) (Trainotti and Stambuk, 2001). Under these conditions, yeast cells undergo morphological changes and produce higher concentrations of glycerol, acetaldehyde and/or acetic acid (Tamas and Hohmann, 2003; Donkin *et al.,* 2010). It may also lead to a decrease in cell health and a delay in the onset of, and completion of AF (Trainotti and Stambuk, 2001; Donkin *et al.,* 2010). The reduction in yeast cell viability with an increase in NaCl level indicates that NaCl toxicity is involved to some extent (Donkin *et al.,* 2010). Although *Oenococcus oeni* seems to be quite tolerant to high NaCl levels in wine, further research is required (Donkin *et al.,* 2010).

Winery wastewater contains large populations of microorganisms, ranging from $10^5$ to $10^8$ colony forming units per millilitre (cfu/mL) (Jourjon *et al*., 2005). The dominant yeast species are *Saccharomyces cerevisiae*, *Candida intermedia, Hanseniaspora uvarum* and *Pichia membranaefaciens* (Malandra *et al.,* 2003). Winery wastewater also contains large LAB and acetic acid bacteria (AAB) populations (Jourjon *et al*., 2005). Therefore, if contact is made between winery wastewater and grapes during irrigation, some microbes may survive on grape berries and be transferred into grape must and wine. If certain unfavourable microbes are transferred from the wastewater into the must and wine, wine composition and quality may decline.

Winery wastewater has a foul smell due to the conversion of organic compounds to, among others, methane and hydrogen sulphide ($H_2S$) under anaerobic conditions (McCarty, 1964). If

these off-odours are transferred onto or into berries and the resulting wines, it may result in tainted wines. To our knowledge the effect of irrigation using winery wastewater on wine sensory characteristics have not been investigated comprehensively.

Very little is known about the effects of irrigation using augmented winery wastewater on juice and wine composition and quality. In this chapter the effects of irrigation using augmented winery wastewater on juice and wine microbial populations and the ability of inoculated yeast and LAB strains to conduct AF and MLF, will be reported. The transfer of elements, applied by irrigation using augmented winery wastewater, into wine will also be shown. Furthermore, chemical composition and sensorial characteristics of bottled wines will be evaluated to see if any alterations occurred as a result of irrigation using various concentrations of augmented winery wastewater.

## 4.2 MATERIALS AND METHODS

### 4.2.1 Small scale vinification procedure and sampling

Wines were made from Cabernet Sauvignon/99R grapes in the 2011 and 2012 vintages according to the standard red winemaking procedure used by the ARC Infruitec-Nietvoorbij experiment cellar, Stellenbosch. Wines were made from grapes of each of three replications of all treatments. Approximately 40 kg of grapes per replication were used for small scale vinification. Grapes were crushed and transferred to 50 L plastic buckets. The crusher was washed with sodium hydroxide (NaOH) and water in between the crushing of the grapes from different experimental plots to avoid cross contamination. After crushing, homogeneous samples were analysed for determination of juice total soluble solids (TSS), pH, total titratable acidity (TTA), ion [nitrogen (N), phosphorus (P), K, Na, Cl, calcium (Ca), magnesium (Mg)] and heavy metal [chromium (Cr), lead (Pb), mercury (Hg), cadmium (Cd), arsenic (As)] content, as well as juice microbial composition. After sampling total sulphur dioxide ($SO_2$) content was adjusted to 20 mg/L. One hour skin contact was allowed before inoculation with rehydrated pure wine yeast (VIN 13, Anchor Yeast, Cape Town, South Africa) at 30 g/hL. Diammonium phosphate (DAP) was added one day after inoculation at 50 g/hL as yeast nutrition. Alcoholic fermentation was conducted on the skins at 25 ˚C during which the skin caps were punched down twice a day to ensure sufficient skin contact and extraction. Alcoholic fermentation was monitored for reducing sugar (RS), ethanol and pH by means of infrared spectroscopy using the Winescan FT 120 instrument (FOSS Analytical A/S software version 2.2.1) and by microbial enumeration of the inoculated yeast strain. Bacterial populations will also be monitored by means of enumeration. Must was fermented to between 0 degree balling (°B) and 2°B after which skins were separated and pressed at *ca.* 0.2 MPa. Press wine and free run wine were combined after which wines were fermented to dryness at 25 °C. After completion of AF, wines

were inoculated with a MLF starter culture (Enoferm Alpha, Lallemand, Stellenbosch, South Africa) to induce MLF. Through the course of MLF, malic acid, lactic acid, pH and VA were monitored using the Winescan FT 120 instrument and the Konelab 20XT instrument, whereas microbial enumeration of the inoculated LAB strain was performed. After completion of MLF, wines were racked and total $SO_2$ adjusted to 85 mg/L. Thereafter, wines were cold stabilised for two weeks at 0°C. Wines were filtered using sterile mats as well as 0.45 µm membranes. Nitrogen filled bottles were used for bottling at room temperature after which $SO_2$ content was adjusted to at least 85 mg/L if necessary. Wines were stored at 14°C prior to sensorial evaluation and chemical analysis, carried out in early September for both seasons.

### 4.2.2 Microbial enumeration

Microbial populations were monitored to identify and monitor the natural microbial flora present in irrigation water, grape must and wine. Furthermore, the possibility that microorganisms are transferred from irrigation water into the must and wine was also investigated. In addition, the effectiveness of the inoculated commercial cultures was evaluated to determine whether irrigation using augmented winery wastewater had an effect on growth and performance of inoculated strains. All microbial counts were determined by plating out 100 µL of a tenfold dilution series (made in sterile water) of irrigation water, must and wine on selective media.

The natural microbial flora of irrigation water was determined by plating out on yeast extract agar. Yeast extract agar plates consisted of 3 g/L yeast extract powder (Biolab, Merck, Wadeville, Gauteng), 5 g/L peptone (Fluka analytical, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and 15 g/L bacteriological agar (Biolab, Merck, Wadeville, Gauteng). The pH of yeast extract agar plates was adjusted to 7.2 with potassium hydroxide (KOH). Yeast extract agar plates were incubated aerobically at 30 ˚C for 3-7 days.

The natural microbial flora of the juice was determined by plating out on YPD-, WLN-, MRST-, MRS- and GYC agar. The YPD plates consisted of 70 g/L yeast peptone dextrose agar (Biolab, Merck, Wadeville, Gauteng). The WLN media consisted of 77 g/L Wallerstein nutrient agar (Fluka analytical, Sigma-Aldrich Chemie GmbH, Steinheim, Germany). The YPD- and WLN media both contained 50 mg/L chloramphenicol (Sigma Aldrich, China) to suppress the growth of LAB and 25 mg/L kanamycin sulphate (Roche Diagnostics G.M.B.H., Mannheim, Germany) to suppress the growth of AAB. The MRST plates consisted of 50 g/L De Man, Rogosa and Sharpe (MRS; Biolab, Merck, Wadeville, Gauteng) and 20 g/L bacteriological agar (Biolab, Merck, Wadeville, Gauteng) supplemented with 10% preservative free tomato juice (All Gold, South Africa), with pH adjusted to 5.0 with hydrochloric acid (HCl). The MRS plates consisted of 50 g/L MRS broth (Biolab, Merck, Wadeville, Gauteng) and 15 g/L bacteriological agar (Biolab, Merck, Wadeville, Gauteng). Both MRST and MRS plates contained 50 mg/L Delvocid Instant

(DSM Food Specialties, The Netherlands) to suppress yeast growth and 25 mg/L kanamycin sulphate to suppress the growth of AAB. The GYC media did not contain any antibiotics as its purpose was to determine total bacterial numbers, including LAB and AAB. The YPD- and WLN media were used to determine the natural yeast flora in the juice and to monitor the growth and survival of the inoculated yeast strain during AF. The MRST- and MRS media were used to determine the natural bacterial flora, with the exception of AAB, in the juice and to monitor bacterial survival and alterations during AF. The MRST media was also used to monitor growth and survival of the inoculated *O. oeni* strain during MLF. The first samples were plated out after inoculation had been completed. The GYC media was used to determine the total natural bacterial flora of juice and to monitor growth and survival during AF. Agar plates were incubated at 30 ˚C for 6-14 days after which plates were counted and cfu/mL were determined. All plates were incubated aerobically. All microbial enumeration data are displayed as an average of the triplicate treatments.

### 4.2.3 Fermentation performance

4.2.3.1 FT-IR spectral measurements

Juice and wine composition was monitored throughout the course of AF and MLF by means of Fourier Transform Mid Infrared (FT-MIR) spectroscopy as described by Malherbe (2007). The Winescan FT120 instrument was used to quantify chemical data that included: reducing sugar (RS), ethanol, pH and VA. The chemical data were predicted from infrared spectra by commercial calibrations or in-house adjustments using the Winescan FT120 2001 version 2.2.1 software.

4.2.3.2 Konelab 20XT instrument.

Homogeneous wine samples were analysed throughout the course of MLF for malic acid and lactic acid using the Konelab 20XT (Thermo Electron Oy, Finland) instrument. The instrument uses enzymatic kits (Enzytec™ *Fluid* L-Malic acid Id-N°: 5280 and Enzytec™ *Fluid* L-Lactic acid Id-N°: 5260, Thermo Fisher Scientific Oy, Finland) for determination of malic acid and lactic acid. Acids were expressed as g/L. Analyses were done according to manufacturer instructions.

### 4.2.4 Wine characteristics

Wet chemistry analysis of bottled wines was performed by a commercial laboratory (Koelenhof, Stellenbosch) according to standard procedure unless otherwise specified.

#### 4.2.4.1 Alcohol

Alcohol concentration was determined by means of pycnometry using a distillation unit (Glasschem, Cape Town, SA), as described by the South African Wine Laboratories Association (2003), and expressed as %v/v.

#### 4.2.4.2 Reducing sugar

Reducing sugar was determined according to Fehling's method, using an automatic titrator (Mettler Toledo Autotitrator, DL22, Microcept, Cape Town, South Africa), according to the method described by the South African Wine Laboratories Association (2003) and expressed as g/L sugar.

#### 4.2.4.3 Glucose

Glucose was determined using glucose enzymatic kits (Boehringer Mannheim, Roche) and expressed as g/L glucose.

#### 4.2.4.4 Fructose

Fructose was determined using fructose enzymatic kits (Boehringer Mannheim, Roche) and expressed as g/L fructose.

#### 4.2.4.5 Free amino nitrogen

Free amino nitrogen was determined according to the Formol titration method, as described by the South African Wine Laboratories Association (2003). Formaldehyde (37%) was adjusted to pH 8.5 using 1 N sodium hydroxide (NaOH). An excess of adjusted formaldehyde was then added to the samples (50 mL). Ten minutes reaction time was allowed before samples were re-titrated to the endpoint of pH 8.5 using 1 N NaOH (Value x 28 = Free amino nitrogen (FAN) expressed as mg/L).

#### 4.2.4.6 pH

For pH determination, an automatic titrator (Mettler Todelo Autotitrator, DL22, Microcept, Cape Town, South Africa) with a combination electrode and temperature probe was used. The electrode was calibrated using certified buffers (Crison pH 7.00 and pH 4.00, Lasec, Cape Town, SA). The pH was determined as described by Iland *et al.* (2000).

#### 4.2.4.7 Total titratable acidity

Total titratable acidity was measured by means of potentiometric titration using an automatic titrator (Mettler Toledo Autotitrator, DL22, Microcept, Cape Town, South Africa). Samples were titrated to the endpoint (pH 7.00) using standardised 0.33 N sodium hydroxide (Merck, Cape

Town, SA). The TTA was determined as described by Iland *et al.* (2000) and expressed as g/L tartaric acid.

4.2.4.8 Tartaric acid

Tartaric acid was determined using tartaric acid enzymatic kits (Boehringer Mannheim, Roche) and expressed as g/L tartaric acid.

4.2.4.9 Malic acid

Malic acid was determined using malic acid enzymatic kits (Boehringer Mannheim, Roche) and expressed as g/L malic acid.

4.2.4.10 Volatile acidity

Volatile acidity was determined by means of a VA still (Glasschem, Stellenbosch, South Africa) and expressed as g/L acetic acid. The method described by the SA Wine Laboratories Association (2003) was used.

4.2.4.11 Colour, phenolics and tannins

Wine colour, phenolics and degree of red pigment colouration were all determined as described by Iland *et al.* (2000). All these parameters were determined spectrophotometrically using a LKB Biochrom Ultrospec IIE spectrophotometer (LKB Biochrom Ltd, Cambridge, U.K.).

*Brown and red colour pigments and colour density:* Wine red and brown colour hue was determined by measuring wine absorbance at $A_{520\ nm}$ and $A_{420\ nm}$ respectively, using 1 mm quartz cuvettes. Red wine colour density was determined by adding the values at $A_{520\ nm}$ and $A_{420\ nm}$. A value of 0-6 represents a light red coloured wine, 6-10 a medium red coloured wine and >10 a dark red coloured wine.

*Total phenolics:* Wine total phenolics were determined spectrophotometrically at $A_{280\ nm}$. The sample (0.1 mL) was added to 10 mL 1N HCl and mixed well. A three hour reaction period was allowed before measuring absorbance at $A_{280\ nm}$.

*Tannins:* Wine tannin composition was determined according to the LA method, developed by Ribéreau-Gayon and Stonestreet (1966), as described by Ribéreau-Gayon *et al.,* 2006.

*Degree of red pigment colouration:* The degree of red pigment colouration (%) was determined spectrophotometrically according to the formula: $A_{520}/A_{520+HCl}$ x 100 (Iland *et al.,* 2000).

4.2.4.12 Ion composition

Wine phosphorus and ion content was analysed by a commercial laboratory (Bemlab, Strand). For determination of P, Na, K, Cl and $SO_4^{2-}$, samples were prepared and analysed according to methods described by Clesceri *et al.* (1998) using an ICP-OES spectrometer (PerkinElmer Optima 7300 DV, Waltham, Massachusetts, USA.).

### 4.2.5 Sensorial characteristics

Sensorial wine evaluation was performed on all wines from all triplicates of all treatments in both seasons. An expert panel consisting of at least 11 judges evaluated the wines. Wines were scored on the occurrence of off-flavours (off-tastes and off-odours) and other atypical characteristics, including: wine colour, overall intensity, vegetative character, berry character, spicy character, acidity, body, astringency and overall quality. Wines were scored by means of a 100 mm unmarked line scale. Each line scale was measured and the mean distance used to determine the degree of presence/liking/disliking by the tasters.

### 3.2.6 Statistical analysis

Microsoft® Excel (Microsoft Corporation, USA) was used to sort raw data and to calculate the standard deviation from the means. Data were subjected to an analysis of variance (ANOVA) by using both, Statistica version 10 (Statsoft, USA) and Statgraphics® (StatPoint Technologies Inc., USA). Significant differences were expressed using 95% confidence intervals.

## 4.3 RESULTS AND DISCUSSION

### 4.3.1 Microbial enumeration

4.3.1.1 Yeast counts in must and during alcoholic fermentation

Results obtained in this study showed that total must yeast cell counts on YPD agar displayed small variation between treatments in the 2011 vintage (Fig 4.1), ranging from $1.8 \times 10^5$ to $3.28 \times 10^5$ cfu/mL. Must yeast counts for the 2012 vintage were very similar amongst all treatments (Fig 4.2), ranging from $5.5 \times 10^5$ to $8.0 \times 10^5$ cfu/mL. Yeast counts on WLN media in the 2012 vintage confirmed the results (Fig 4.3). As variation between treatments within the 2011 vintage could not be associated with any treatment trends, the differences were probably due to naturally occurring varying conditions within the vineyard. Therefore, irrigation using these various concentrations of augmented winery wastewater did not affect the total yeast microbial flora in the grape must. The foregoing indicates that yeast cells were probably not transferred from augmented winery wastewater onto grape berries and into must, to a noteworthy level. As identification of yeast strains was beyond the scope of this study, the transfer of yeast cells from

irrigation water to grape must and wine cannot be discussed in depth and should be investigated further.

Yeast species, even though not as ubiquitous as bacteria species occur widespread throughout nature (Phaff *et al.,* 1978). They are non-motile and therefore rely on aerosols, human activity and animal vectors for their natural dispersal (Walker, 1998). Therefore, yeast cells may be transferred from the winery wastewater onto grapes by means of wind or vectors during irrigation. The yeast microflora of grapes is highly variable and usually dominated by low alcohol-tolerant species of the genera *Kloeckera*, *Hanseniaspora* and *Candida* (Fleet and Heard, 1993). Total yeast cell counts in grape must for both, the 2011 and 2012, vintages were within norms for South African grape musts. Jolly *et al*. (2003) reported that total non-*Saccharomyces* cell counts for Chardonnay must from four regions in the Western Cape over three seasons, varied between 8.6 x $10^3$ and 5.2 x $10^6$ cfu/mL. Di Maro *et al.* (2007) found mean must yeast counts on YPD agar for Catalanesca must in Italy to be 4.35 x $10^6$ cfu/mL.

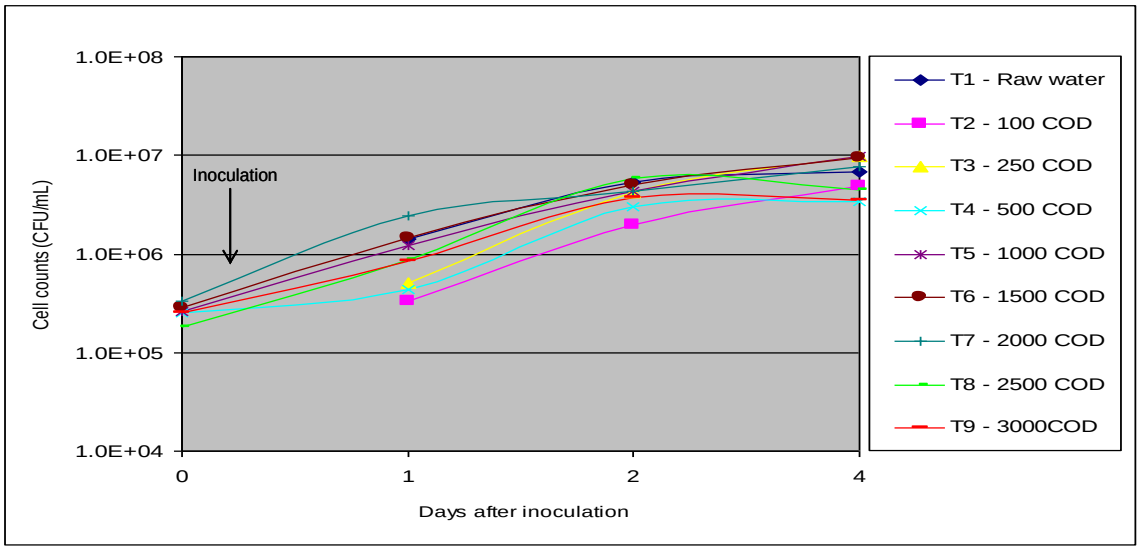


**Fig 4.1** Yeast cell counts (cfu/mL) on YPD media in the must and during alcoholic fermentation for wines made from grapes of Cabernet Sauvignon/99R grapevines, irrigated using augmented winery wastewater, in the 2011 vintage. Each timepoint represents the average of triplicate treatments.

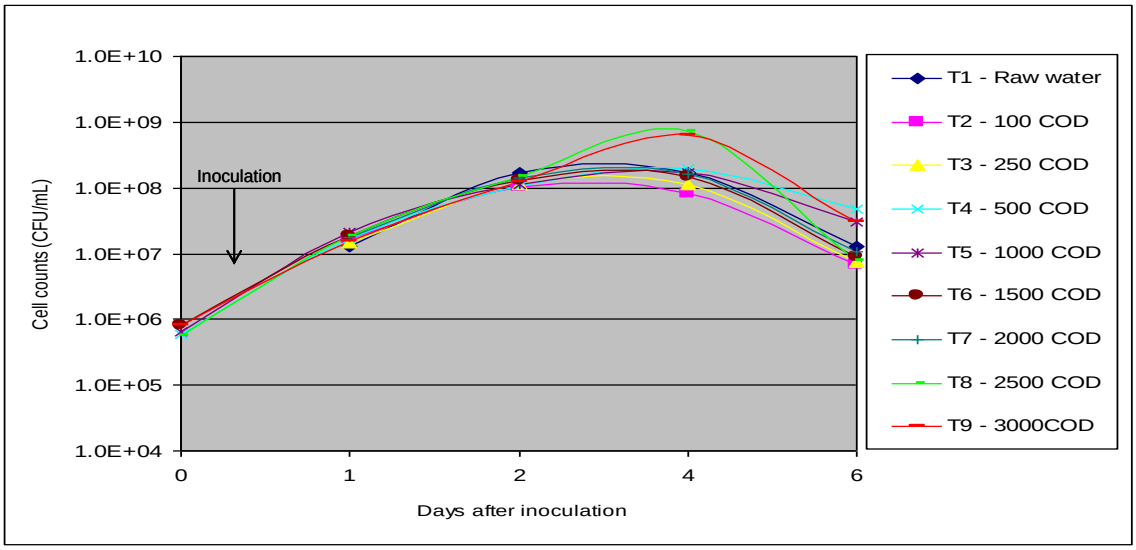


**Fig 4.2** Yeast cell counts (cfu/mL) on YPD media in the must and during alcoholic fermentation for wines made from grapes of Cabernet Sauvignon/99R grapevines, irrigated using augmented winery wastewater, in the 2012 vintage. Each timepoint represents the average of triplicate treatments.

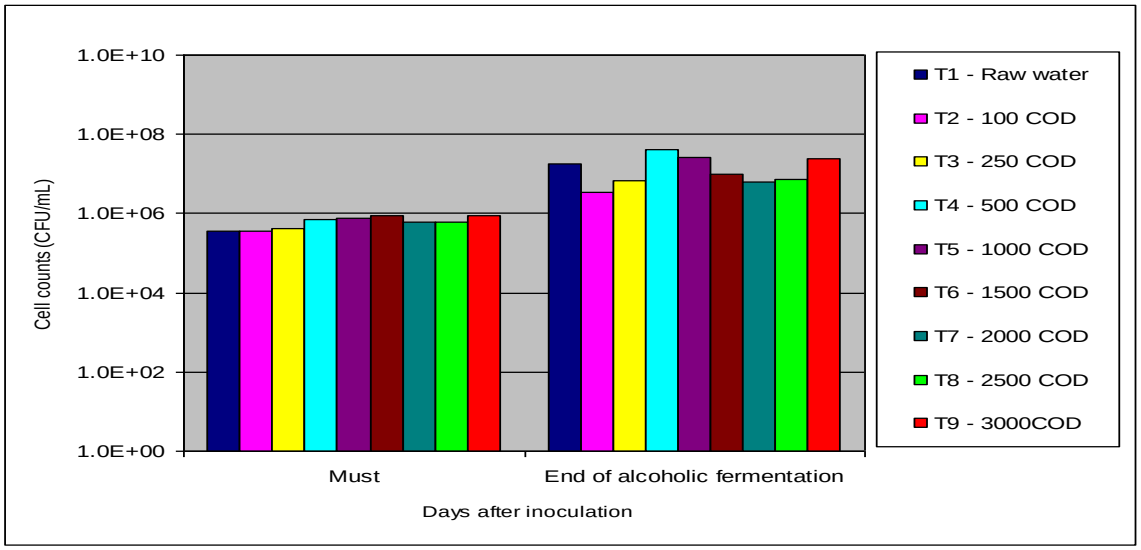


**Fig 4.3** Yeast cell counts (cfu/mL) on WLN media in the must and at the end of alcoholic fermentation for wines made from grapes of Cabernet Sauvignon/99R grapevines, irrigated using augmented winery wastewater, in the 2012 vintage. Each timepoint represents the average of triplicate treatments.

Following inoculation, total yeast cell counts increased considerably during the course of AF (Figs 4.1 & 4.2), ranging from 3.4 x $10^5$ to 9.9 x $10^6$ cfu/mL in the 2011 vintage and 7.8 x $10^6$ to 7.1 x $10^8$ cfu/mL in the 2012 vintage. Total yeast cell counts for all treatments, during the 2011 vintage, increased during the course of AF. In the 2012 vintage, yeast cell counts increased during the earlier stages of AF, but reached a plateau as the cells reached a maximum number due to competition for nutrients and an increase in ethanol concentration. Thereafter, yeast cell populations decreased as the ethanol concentration further increased and the available sugar and nutrients decreased. The continual increase in yeast population, noticed for the 2011 vintage, was probably because AF had not been completed at the time when the yeast count

was determined on day four. Therefore, cell numbers may have decreased after this point as competition for nutrients as well as ethanol concentration was increased. Nevertheless, no trends were observed with regards to treatments. The higher yeast cell counts for T8 & T9 on day four in the 2012 vintage was probably due to human error while plating out, as counts for T8 and T9 are similar to all other treatments during the other sampling periods. Therefore, irrigation using augmented winery wastewater at various COD concentrations did not affect the composition and suitability of the wine medium for growth and survival of the inoculated *S. cerevisiae* yeast strain. Similar to YPD media, yeast cell counts on WLN media (Fig 4.3), for the 2012 vintage, further demonstrated that higher numbers of yeast cells were present at the end of AF than in the must. Furthermore, yeast cell counts for both mediums indicated that *S. cerevisiae* dominated AF following its inoculation. This was in accordance with literature which states that the ethanol tolerant *S. cerevisiae* is expected to be the dominant yeast strain once juice starts fermenting and ethanol concentration increases (Fleet and Heard, 1993).

4.3.1.2 Bacterial counts in must and during alcoholic fermentation

GYC media counts showed the total bacterial flora in must and during AF (Figs 4.4 & 4.5). Total counts on MRST- and MRS media showed the total LAB counts in must and during AF (Figs 4.6 - 4.9). Lactic acid bacteria were the dominant bacteria present in the must, expectedly as they are the dominant wine-associated bacteria. Wine-associated LAB belongs to the taxonomic genera *Leuconostoc, Pediococcus* and *Lactobacillus* (Buchanan and Gibbons, 1974; London, 1976; Stamer, 1979; Irwin *et al.,* 1983). As total bacterial counts on all three these media were very comparable, AAB did not appear to be present in large numbers. Therefore, AAB probably did not contribute to wine composition during AF. Bacteria cells were present in significant numbers in musts from all treatments. However, no definite treatment trends were observed. Furthermore, bacterial numbers in irrigation water did not necessarily increase as the level of COD in the augmented winery wastewater increased, as shown in Chapter 3 (Fig 3.4). For these reasons, grape must bacterial flora was not affected by irrigation using augmented winery wastewater. Figure A2 in Addendum A display the similarity in LAB flora of must from grapevines irrigated with raw water and two wastewater irrigation treatments. Figure A3 in Addendum A indicate the similarity in total bacterial flora of must from grapevines irrigated with raw water and two wastewater irrigation treatments.

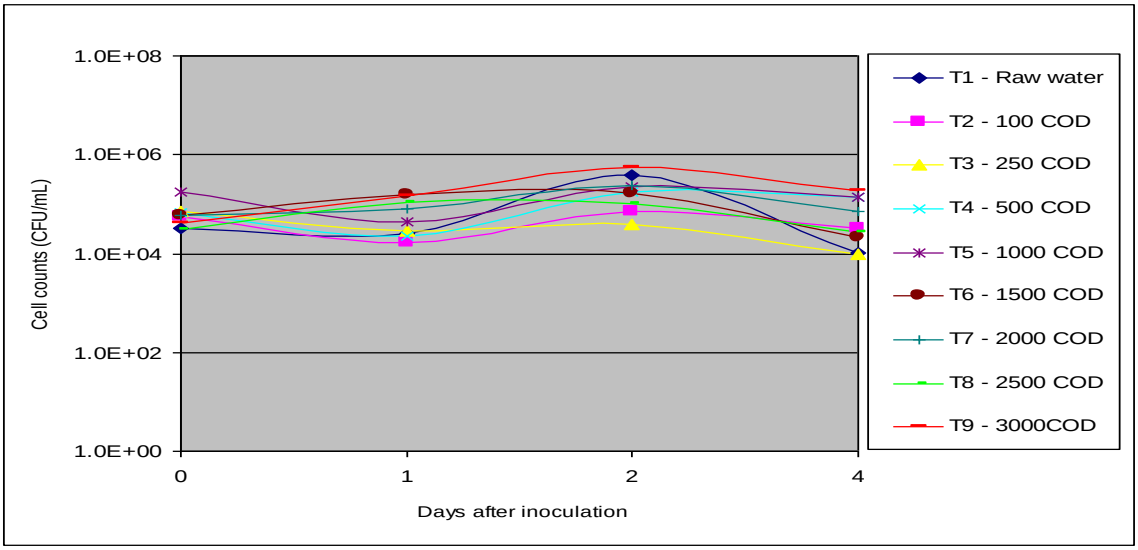


**Fig 4.4** Total bacterial counts (cfu/mL) on GYC media in the must and during alcoholic fermentation for wines made from grapes of Cabernet Sauvignon/99R grapevines, irrigated using augmented winery wastewater, in the 2011 vintage. Each timepoint represents the average of triplicate treatments.

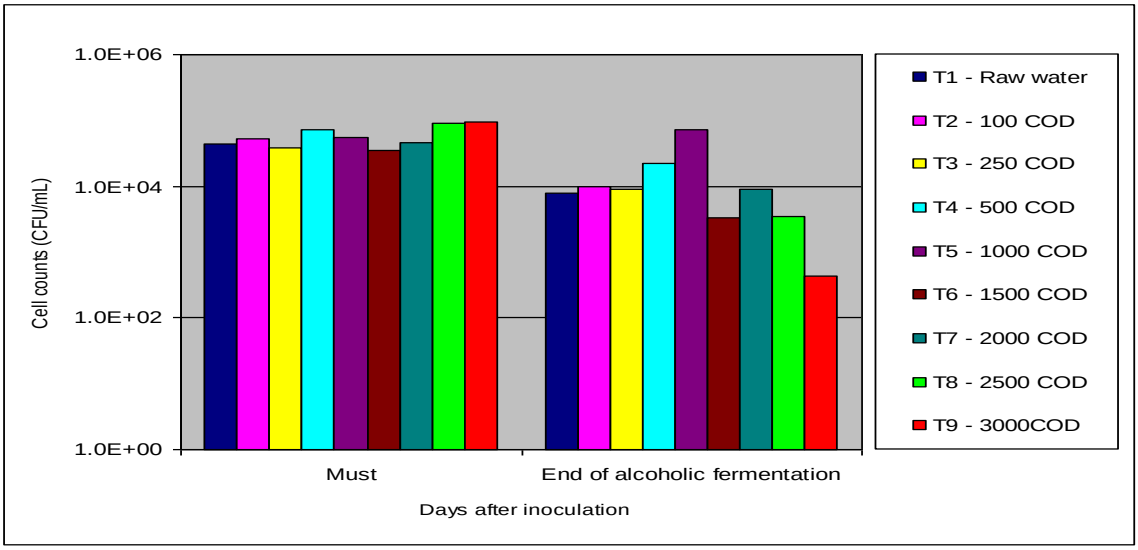


**Fig 4.5** Total bacterial counts (cfu/mL) on GYC media in the must and at the end of alcoholic fermentation for wines made from grapes of Cabernet Sauvignon/99R grapevines, irrigated using augmented winery wastewater, in the 2012 vintage. Each timepoint represents the average of triplicate treatments.

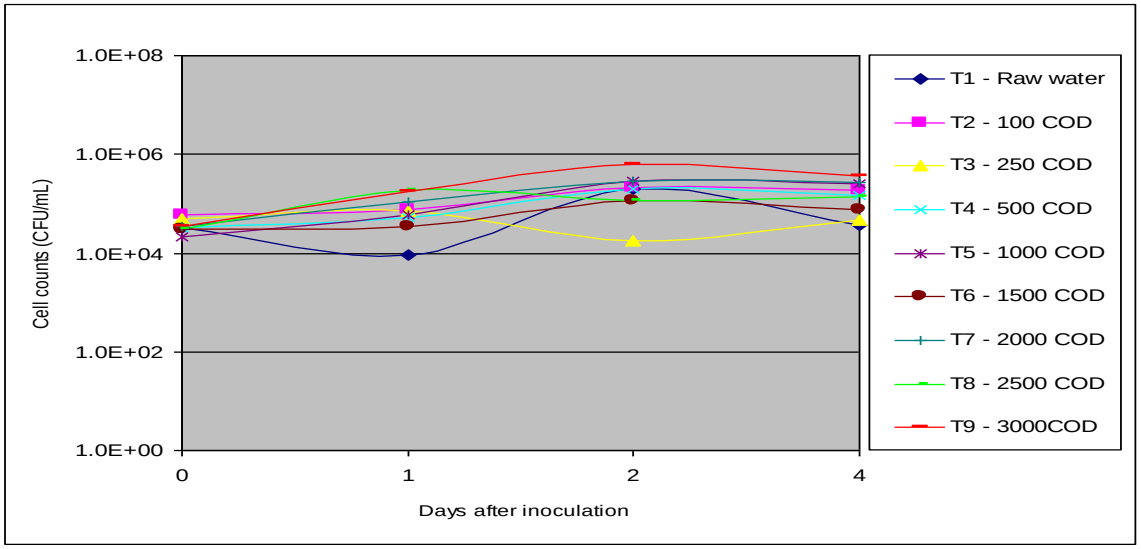


**Fig 4.6** Total LAB counts (cfu/mL) on MRST media in the must and during alcoholic fermentation for wines made from grapes of Cabernet Sauvignon/99R grapevines, irrigated using augmented winery wastewater, in the 2011 vintage. Each timepoint represents the average of triplicate treatments.

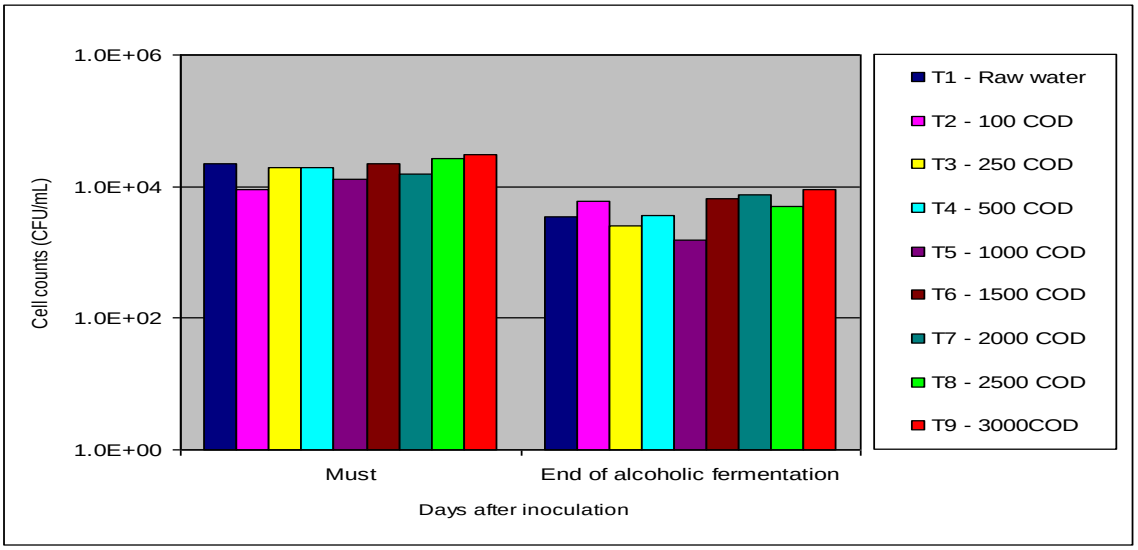


**Fig 4.7** Total LAB counts (cfu/mL) on MRST media in the must and at the end of alcoholic fermentation for wines made from grapes of Cabernet Sauvignon/99R grapevines, irrigated using augmented winery wastewater, in the 2012 vintage. Each timepoint represents the average of triplicate treatments.

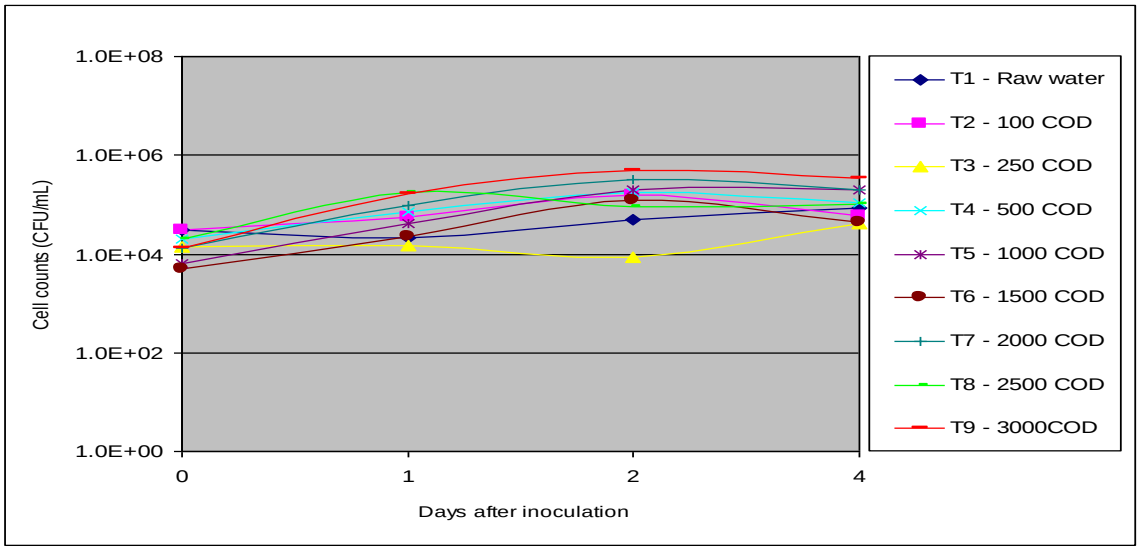


**Fig 4.8** Total LAB counts (cfu/mL) on MRS media in the must and during alcoholic fermentation for wines made from grapes of Cabernet Sauvignon/99R grapevines, irrigated using augmented winery wastewater, in the 2011 vintage. Each timepoint represents the average of triplicate treatments.

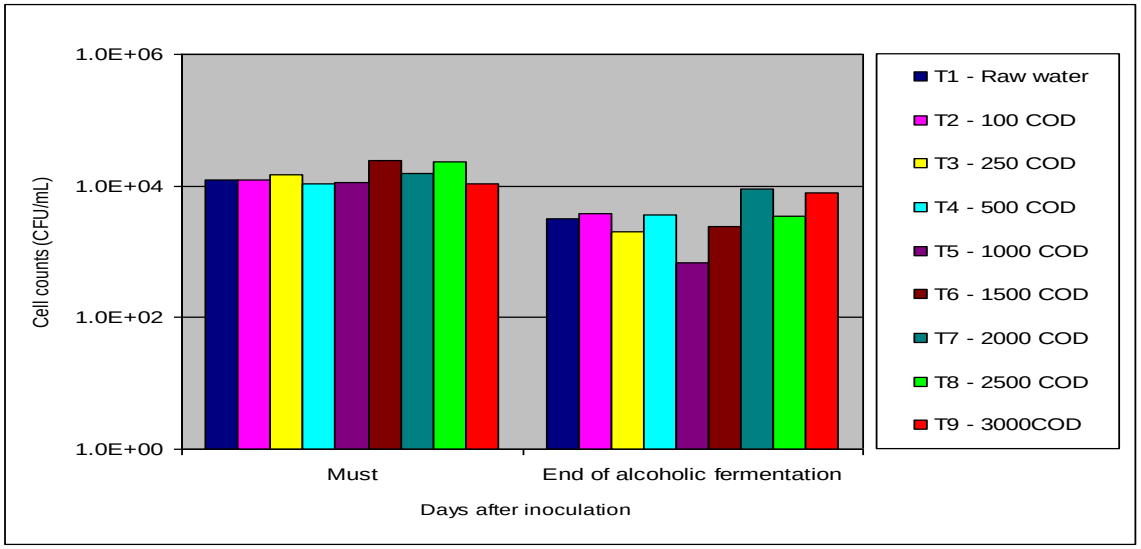


**Fig 4.9** Total LAB counts (cfu/mL) on MRS media in the must and at the end of alcoholic fermentation for wines made from grapes of Cabernet Sauvignon/99R grapevines, irrigated using augmented winery wastewater, in the 2012 vintage. Each timepoint represents the average of triplicate treatments.

Total bacterial counts on GYC media remained at more or less constant levels in the 2011 vintage, with slight increases for some treatments and slight decreases for others, as AF progressed. In the 2012 vintage, bacteria were present in fewer numbers by the end of AF than in the must. This decrease could probably be attributed to an increase in fermentation metabolites, including alcohol. Lactic acid bacteria counts on MRST- and MRS media increased during the course of AF, in the 2011 vintage. In the 2012 vintage, however, these counts decreased in the must towards the end of AF. Again, this was probably due to an increase in

fermentation metabolites such as alcohol. The total amount of bacteria, present during AF, was large enough that they could have contributed to wine composition and quality. Furthermore, even though bacterial counts showed considerable variation between treatments, no definite treatment trends were observed for any of the three growth media. Therefore, irrigation using augmented winery wastewater did not affect the composition of grape must and wine in such a manner that the growth and survival of bacteria was affected during AF. The identification of specific bacteria strains was beyond the scope of this study. Therefore, the specific bacteria strains that were present during AF cannot be discussed. Furthermore, the possibility that some of these individual bacterial species were affected by irrigation using winery wastewater, cannot be discussed and should be investigated further.

4.3.1.3 Lactic acid bacteria counts during malolactic fermentation

Following inoculation with MLF starter culture (Enoferm Alpha, Lallemand, Stellenbosch, South Africa), *O. oeni* cell counts for wines made from all nine treatments ranged between 9.0 x $10^5$ and 1.3 x $10^6$ cfu/mL in the 2011 vintage and 2.0 x $10^6$ and 2.7 x $10^6$ cfu/mL in the 2012 vintage (Figs 4.10 & 4.11), just about reaching the target minimum cell population of 1.0 x $10^6$ cfu/mL. *O. oeni* is the dominant LAB species present in wine as it is best adapted to survive in the wine environment (Wibowo *et al.,* 1985). Furthermore, *O. oeni* is also the preferred strain used as commercial starter culture for conducting MLF (Wibowo *et al*., 1985; Davis *et al.,* 1988; Drici-Cachon *et al.,* 1996; Lonvaud-Funel, 1999). A decline in the viable LAB cell population to below 1.0 x $10^6$ cfu/mL has previously been linked to a reduced ability to complete MLF (Wibowo *et al.,* 1988). Thereafter, *O. oeni* cell populations increased as MLF progressed, reaching cell populations of between 1.1 x $10^7$ and 3.3 x $10^7$ cfu/mL in the 2011 vintage and 1.55 x $10^6$ and 2.6 x $10^7$ cfu/mL in the 2012 vintage. In the 2011 vintage, a large increase in *O. oeni* cells was observed from inoculation to day seven, reaching cell numbers ranging from 1.1 x $10^7$ to 3.2 x $10^7$ cfu/mL by day seven. The rapid growth and high cell populations induced a rapid completion of MLF within seven days for wines made from all treatments (Fig 4.14). A more gradual increase in cells was observed during MLF in the 2012 vintage with a rapid increase only observed from day nine to day 13, reaching cell numbers ranging from 1.2 x $10^7$ to 2.6 x $10^7$ cfu/mL by day 13. Thus, maximum cell counts for the 2012 vintage, was reached after completion of MLF, on day 13. The gradual increase in *O. oeni* cell counts during the 2012 vintage resulted in the longer duration of MLF (Fig 4.15). However, wines from all treatments completed MLF within 13 days, which is still relatively fast.

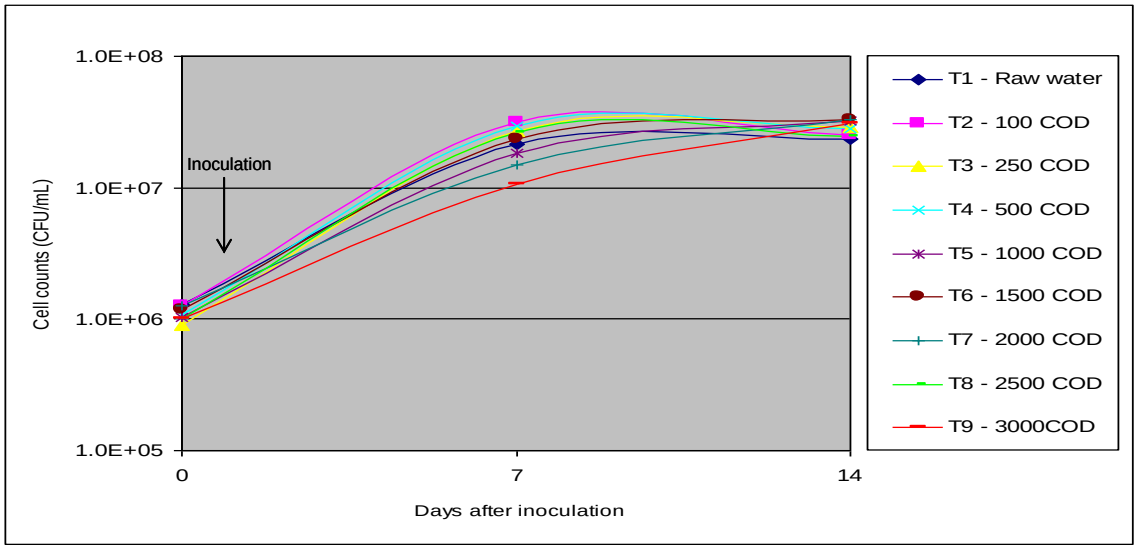


**Fig 4.10** O. *oeni* cell counts (cfu/mL) on MRST media during malolactic fermentation for wines made from grapes of Cabernet Sauvignon/99R grapevines, irrigated using augmented winery wastewater, in the 2011 vintage. Each timepoint represents the average of triplicate treatments.

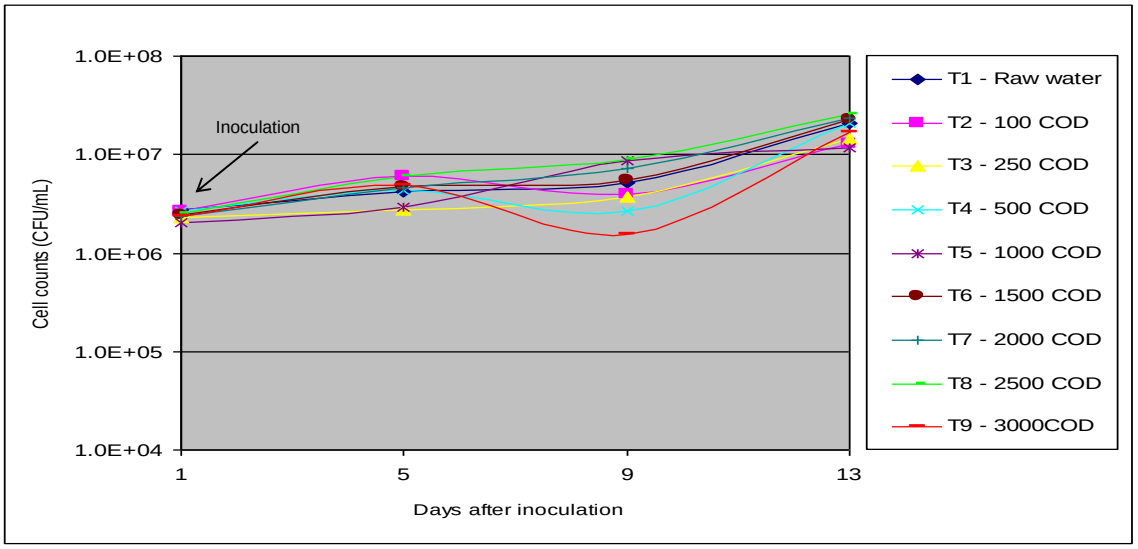


**Fig 4.11** O. *oeni* cell counts (cfu/mL) on MRST media during malolactic fermentation for wines made from grapes of Cabernet Sauvignon/99R grapevines, irrigated using augmented winery wastewater, in the 2012 vintage. Each timepoint represents the average of triplicate treatments.

Even though there was considerable variation in *O. oeni* counts at certain stages during MLF, especially day seven in the 2011 vintage and day nine in the 2012 vintage, no treatment trends were observed in either of the vintages (Figs 4.10 & 4.11). Variation in cell counts could be attributed to naturally occurring variation in the wine medium in which bacteria had to grow and ferment, as well as natural occurring variation when plating out on nutrient mediums. The foregoing indicates that irrigation using augmented winery wastewater did not affect growth and survival of the inoculated LAB strain, nor its ability to conduct and successfully complete MLF. Furthermore, LAB was probably not transferred from any of the wastewater irrigation treatments

into the wine. This was to be expected as bacterial counts for irrigation water treatments that contained higher levels of COD was not necessarily higher than that of the river water treatment (Chapter 3, Fig 3.4).

Irrigation using Na-rich water, such as winery wastewater, may lead to the development of saline soil conditions (Van Schoor, 2005). Grapevines growing in Na- and Cl-rich saline soils may absorb these elements to a large extent and produce juice and wines with increased NaCl concentrations (Stevens and Walker, 2002; Stevens *et al.,* 2011). Even though wine yeast and bacteria are able to survive and proliferate in a wide range of environments, containing high ethanol concentrations and low pH values, their ability to grow, survive and ferment in the wine medium may be inhibited by osmotic stress caused by high juice and wine NaCl levels (Trainotti and Stambuk, 2001). Extremely high levels of juice and wine NaCl (33.59 g/L) have been found to cause a decrease in maximum *S. cerevisiae* yeast cell counts in the order of 25% to 50% (Donkin *et al.,* 2010). Effects were also seen on culture viability where a relative viability in the order of 20% was observed. In addition to impacting on yeast cells, these high NaCl levels may also impact on LAB. Furthermore, since inoculation with LAB starter cultures usually occur after completion of AF, LAB cells will be subjected to metabolites produced during AF as well. Donkin *et* al. (2010) reported that the ability of *O. oeni* to survive, proliferate and conduct MLF, would not be affected at concentrations of NaCl that may be present in wine. However, NaCl concentrations exceeding 12 g/L has been found to have a negative impact on LAB (Le Marrec *et al.,* 2007). It is, however, highly unlikely that these levels of NaCl will ever be present in wines where salt additions have not been made.

A decline in maximum yeast and LAB cell counts was not caused by irrigation using augmented winery wastewater in this study. Therefore, the various wastewater irrigation treatments did not affect the ability of yeast and *O. oeni* cells to survive, proliferate and conduct AF and MLF. Wine Na and Cl concentrations were not increased by irrigation using augmented winery wastewater. In addition, levels were within the recommended norms for wines (Department of Water Affairs and Forestry, 1996; ANZECC, 2000), containing a maximum of 32.8 mg/L for Na and 56.4 mg/L for Cl in either of the two seasons. Therefore, no osmotic stress was caused as a result of irrigation using augmented winery wastewater in this study. Furthermore, even if Na and Cl levels were increased by irrigation using augmented winery wastewater, the levels of NaCl have to be extremely high, 33.59 g/L for yeasts and 12 g/L for LAB, to significant affect microbial growth and survival (Donkin, 2010; Le Marrec *et al.,* 2007). Thus, it is highly unlikely that levels that inhibit the growth of *S. cerevisiae* or *O. oeni*, would be attained due to wastewater irrigation. Evidence suggests that mild increases in wine salt content may even stimulate the tempo and completion of MLF (Rodriguez *et al.,* 1990, Henick-Kling and Park, 1994; Donkin *et al.,* 2010). Donkin *et al.* (2010) reported this value to be 2 g/L NaCl. This stimulatory effect was however

not observed in this study, as wine salt levels were not increased as a result of wastewater irrigation. The stimulatory effect of higher salt concentrations is largely unclear and further research is required on this subject.

Irrigation using augmented winery wastewater resulted in a trend of increasing juice pH in the 2012 vintage (Chapter 3, Table 3.4) and an increase in wine pH in both vintages (Table 4.2). The pH of the wine directly determines the LAB species that survive and proliferate in the wine (Kunkee, 1967). The growth of *O. oeni* is favoured at a pH of 3.5 and lower, while wine pH values of higher than 3.5 generally favour the growth of *Lactobacillus* and *Pediococcus* species (Henick-Kling, 1993). The inoculated *O. oeni* LAB strain completely dominated throughout MLF. As all wines had high pH values (> 3.96), this indicates that *O. oeni* may perform better than expected in high pH wines. All other LAB strains were present at numbers lower than $1.0 \times 10^4$ cfu/mL. Therefore, LAB strains other than the inoculated *O. oeni* strain probably did not make a noteworthy contribution to MLF. Wine pH may also affect the duration and successful completion of MLF by influencing malolactic activity (Henick-Kling, 1993). The highest malolactic activity is found between pH 3.5 and 4.0 (Bauer and Dicks, 2004). The increase in wine pH due to irrigation using augmented winery wastewater may, therefore, result in a shortened duration of MLF as malolactic activity is increased. This increase in wine pH and malolactic activity may be especially advantageous for cool wine producing regions, resulting in fewer complications with the completion of MLF.

### 4.3.2 Monitoring must composition, alcoholic- and malolactic fermentation.

Alcoholic fermentation is the primary fermentation process in winemaking mainly conducted by the yeast, *Saccharomyces cerevisiae*. Malolactic fermentation on the other hand is the secondary fermentation process in winemaking, referring to the degradation of L-malic acid to L-lactic acid and carbon dioxide (Davis *et al.,* 1985). These fermentation processes contribute largely to the composition and final quality of the wine. Therefore, it is important that primary and secondary wine fermentations are conducted under stress free conditions, as off-odours are often produced when the microbes experience some form of stress, to obtain wine of the highest quality.

Alcoholic fermentation for all wines was completed (≤5 g/L RS) within six days in both vintages (Figs 4.12 & 4.13). With the exception of T8 which completed AF within four days in the 2011 vintage, sugar utilization by yeast strain (VIN 13 Anchor Yeast, Cape Town, South Africa) was not affected by the various wastewater irrigation treatments when compared to raw water irrigation. Wines made from T8 had slightly higher yeast cell populations on day 2 (Fig 4.1), which explains its slightly faster fermentation rate. The higher yeast cell number for T8 was not related to a treatment effect, as discussed earlier. Therefore, the increased fermentation tempo

for T8 could not be associated with a wastewater irrigation treatment effect, but rather to a slightly higher inoculation or to naturally occurring variation in yeast cell growth and fermentation tempo. No variation occurred in the rate of fermentation in the 2012 vintage. Ethanol production was related to the tempo of sugar utilization, thus, wines that fermented faster had an accelerated tempo of ethanol production. Therefore, as with sugar utilization, no trends were observed with regards to treatments. Furthermore, total ethanol production was similar for all treatments, suggesting that the sugar-ethanol conversion by the fermenting yeast cells was not influenced by irrigation using augmented winery wastewater.

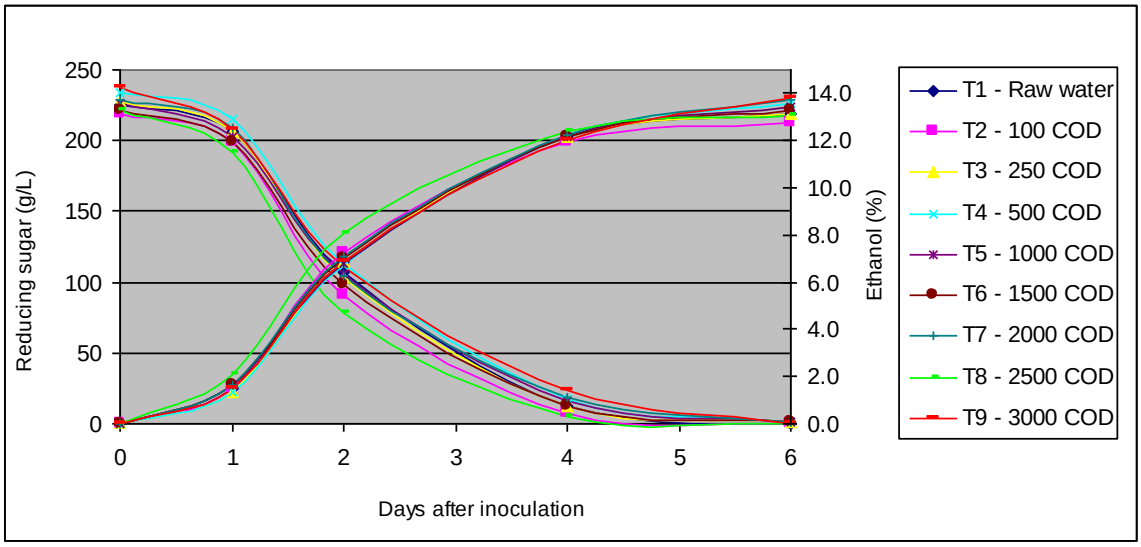


**Fig 4.12** The evolution of reducing sugar (g/L) and ethanol (%) during alcoholic fermentation for wines made from grapes of Cabernet Sauvignon/99R grapevines, irrigated using augmented winery wastewater, in the 2011 vintage. Data shown indicate the average changes in reducing sugar and ethanol for each treatment repeated in triplicate. The RSD is less than 10% between fermentation repeats.

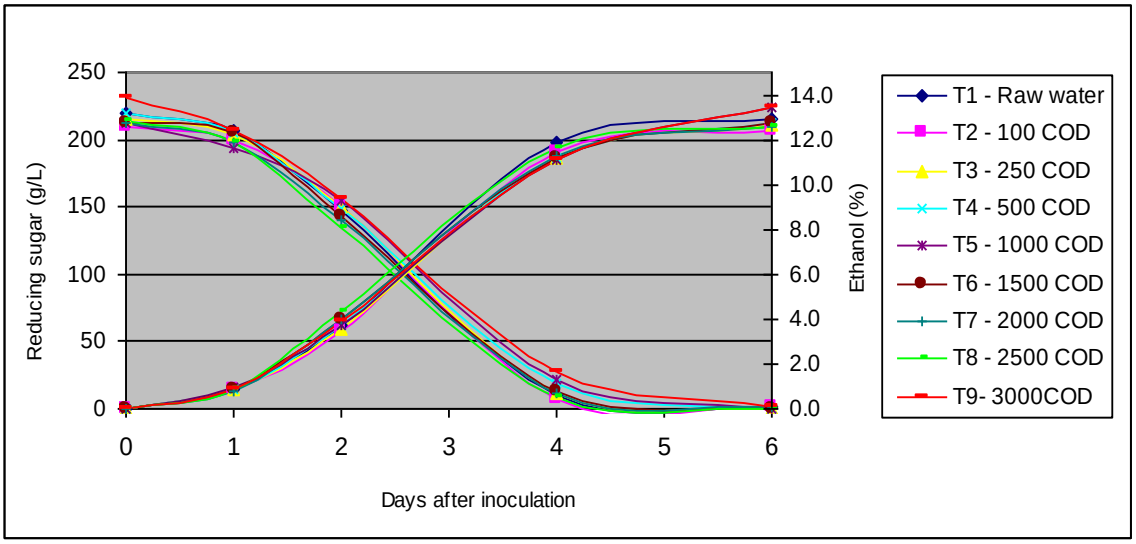


**Fig 4.13** The evolution of reducing sugar (g/L) and ethanol (%) during alcoholic fermentation for wines made from grapes of Cabernet Sauvignon/99R grapevines, irrigated using augmented winery wastewater, in the 2012 vintage. Data shown indicate the average changes in reducing sugar and ethanol for each treatment repeated in triplicate. The RSD is less than 10% between fermentation repeats.

Malic acid for wines made from all treatments was fermented to less than 0.3 g/L within seven days in the 2011 vintage (Fig 4.14) and 13 days in the 2012 vintage (Fig 4.15), following inoculation with *O. oeni* MLF starter cultures. Therefore, indicating the completion of MLF within these respective times. Although malic acid concentrations varied before the onset of MLF in both vintages, no treatment trends were observed. Furthermore, the variation within the 2011 vintage was not consistent with variation within the 2012 vintage. In addition, no treatment trends were observed with regards to tempo of malic acid degradation. Thus, the ability of the *O. oeni* strain to complete MLF was not influenced by any of the wastewater irrigation treatments. As explained under the fore mentioned section, winery wastewater irrigation may lead to elevated juice NaCl levels under certain conditions. Furthermore, elevated juice NaCl levels cause harsh conditions for microorganism cells (Trainotti and Stambuk, 2001). Although a study conducted by Donkin *et al*. (2010) suggested that *O. oeni* is not negatively affected by high juice NaCl levels, care should be taken to ensure the effective and rapid completion of MLF and to avoid the production of unwanted products due to stressful fermenting conditions for the inoculated LAB strain. No treatment trends were observed with regards to lactic acid production in either of the two vintages (Figs 4.14 & 4.15). Therefore, suggesting that the various wastewater irrigation treatments had no effect on lactic acid production by *O. oeni* during MLF.

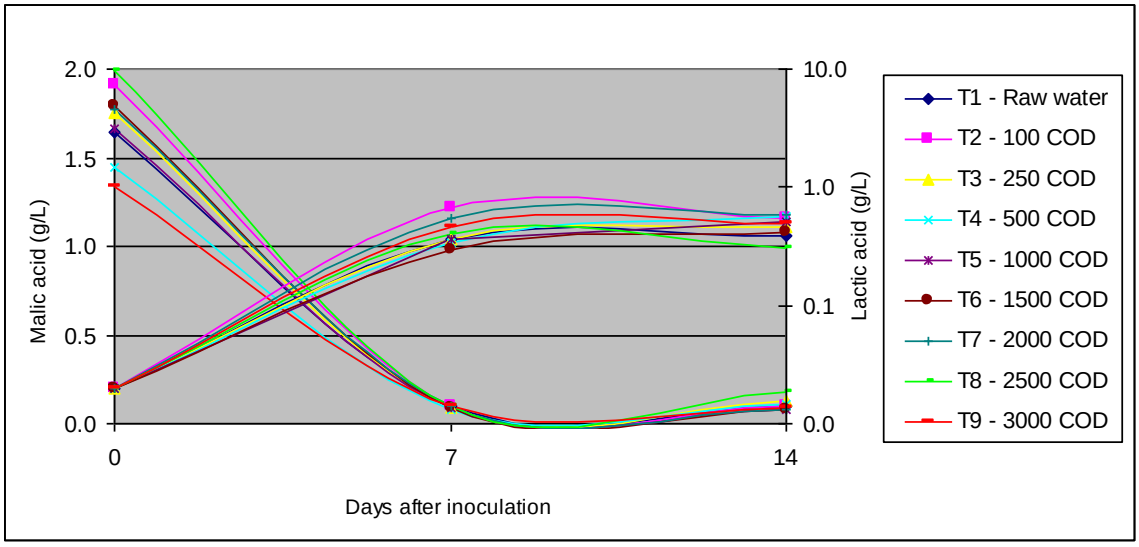


**Fig 4.14** The evolution of malic acid (g/L) and lactic acid (g/L) during malolactic fermentation for wines made from grapes of Cabernet Sauvignon/99R grapevines, irrigated using augmented winery wastewater, in the 2011 vintage. Data shown indicate the average changes in malic acid and lactic acid for each treatment repeated in triplicate.

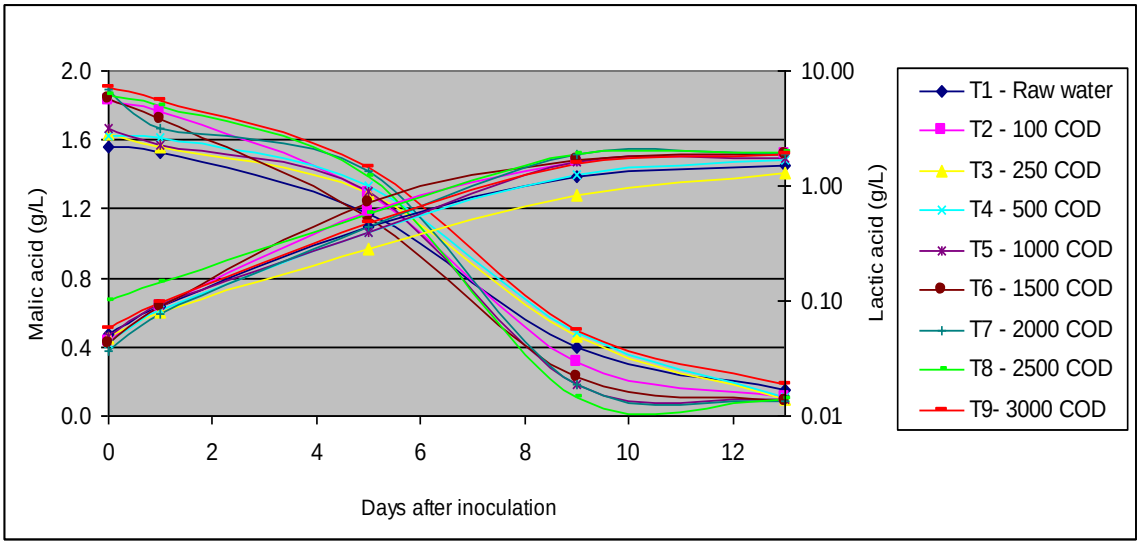


**Fig 4.15** The evolution of malic acid (g/L) and lactic acid (g/L) during malolactic fermentation for wines made from grapes of Cabernet Sauvignon/99R grapevines, irrigated using augmented winery wastewater, in the 2012 vintage. Data shown indicate the average changes in malic acid and lactic acid for each treatment repeated in triplicate.

With the exception of T2, wine pH tended to be higher for the treatments that received irrigation water containing higher levels of COD in both vintages (Figs 4.16 & 4.17). This tendency was observed throughout the course of AF and MLF. The pH of wines made from all treatments increased considerably during MLF. Bousbouras and Kunkee (1971) found that MLF brought about an increase in wine pH as large as 0.2 units. Still, the increased pH for treatments receiving irrigation water with higher COD levels did not affect fermentation performance for either of the key wine-associated fermentation processes. Furthermore, wine pH after bottling was also increased as the level of COD in the augmented winery wastewater increased. Further discussion on the effect of wastewater irrigation on wine pH will follow in the subsequent section.

Volatile acidity increased substantially during MLF in both vintages (Figs 4.18 & 4.19). Generally there is an increase of 0.1 to 0.2 g/L in acetic acid concentration during MLF (Bartowsky and Henschke, 1995), as observed for the 2012 vintage. However, the increase in VA by far exceeded the general increase associated with MLF in the 2011 vintage. This was attributed to the growth of acetic acid bacteria on wines due to the high pH of wines. Even though some variation was observed in VA production during AF and MLF, the amount produced was not related to the level of COD in the augmented winery wastewater. Therefore, irrigation using augmented winery wastewater did not affect the wine matrix in such a manner that VA production by yeast and LAB species was promoted. Volatile acidity of bottled wines will be discussed in the following section.

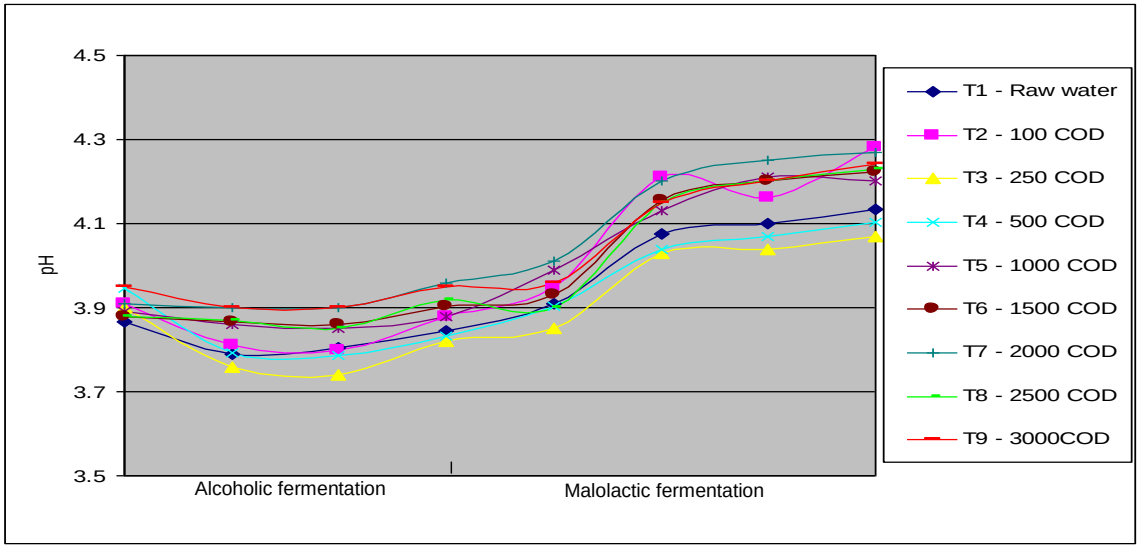


**Fig 4.16** The evolution of pH throughout alcoholic- and malolactic fermentation for wines made from grapes of Cabernet Sauvignon/99R grapevines, irrigated using augmented winery wastewater, in the 2011 vintage. Data shown indicate the average changes in pH for each treatment repeated in triplicate. The RSD is less than 10% between fermentation repeats.

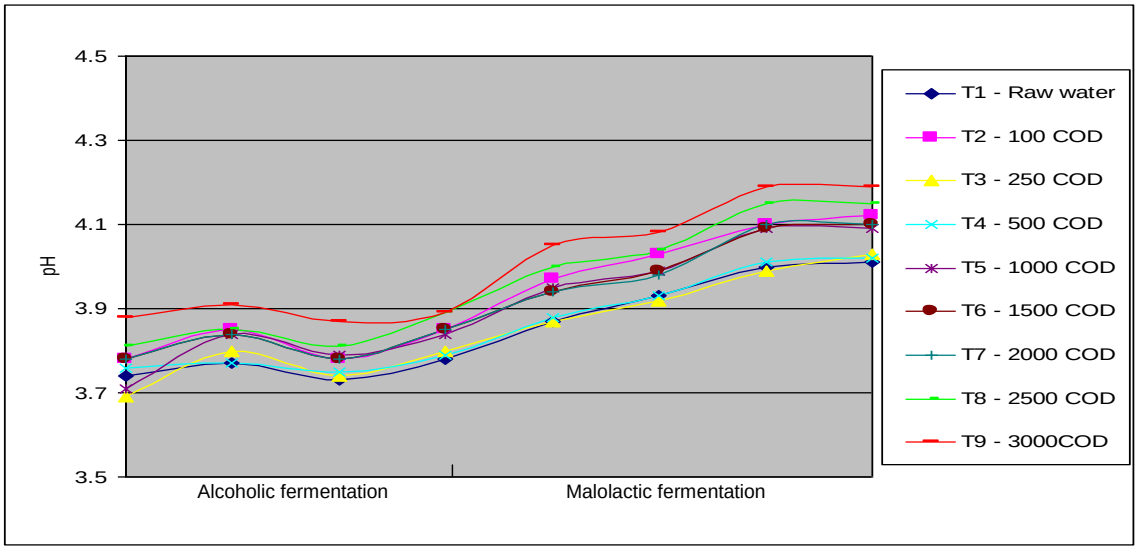


**Fig 4.17** The evolution of pH throughout alcoholic- and malolactic fermentation for wines made from grapes of Cabernet Sauvignon/99R grapevines, irrigated using augmented winery wastewater, in the 2012 vintage. Data shown indicate the average changes in pH for each treatment repeated in triplicate. The RSD is less than 10% between fermentation repeats.

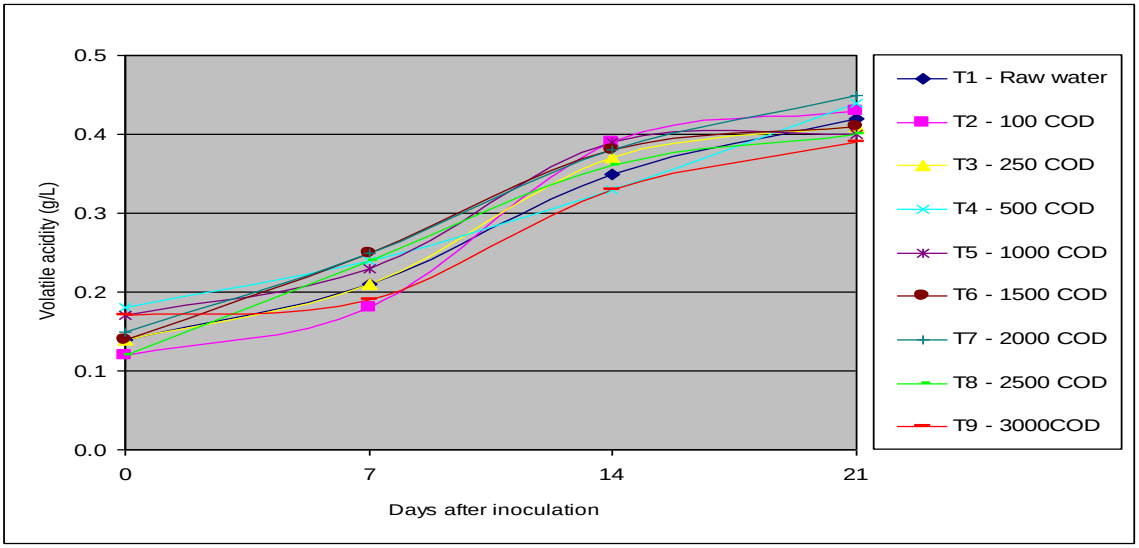


**Fig 4.18** The evolution of volatile acidity (g/L) during malolactic fermentation for wines made from grapes of Cabernet Sauvignon/99R grapevines, irrigated using augmented winery wastewater, in the 2011 vintage. Data shown indicate the average changes in volatile acidity for each treatment repeated in triplicate. The RSD is less than 10% between fermentation repeats.

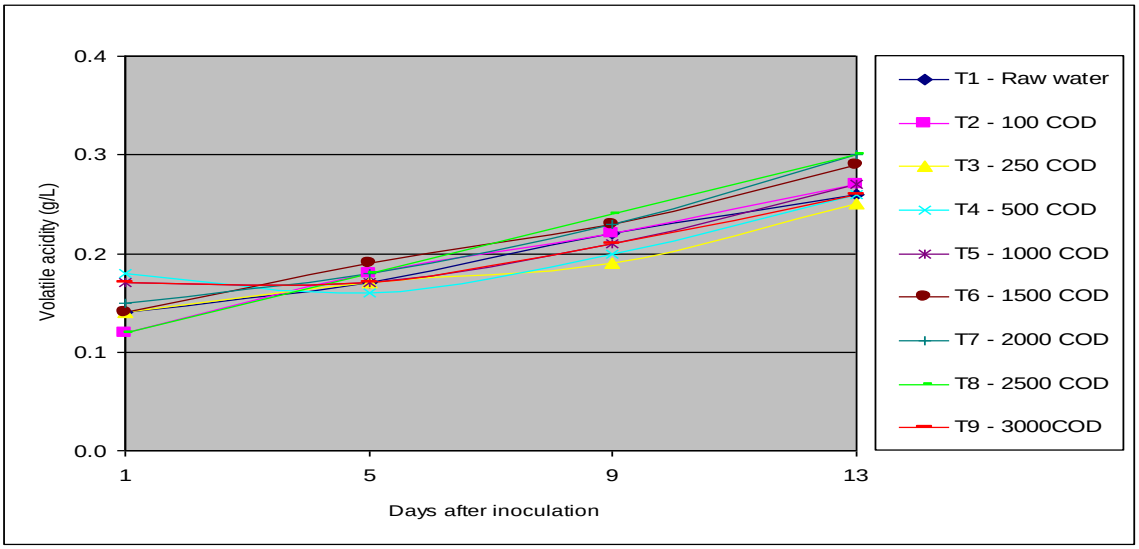


**Fig 4.19** The evolution of volatile acidity (g/L) during malolactic fermentation for wines made from grapes of Cabernet Sauvignon/99R grapevines, irrigated using augmented winery wastewater, in the 2012 vintage. Data shown indicate the average changes in volatile acidity for each treatment repeated in triplicate. The RSD is less than 10% between fermentation repeats.

As explained earlier, if irrigation using winery wastewater causes large increases in juice and wine Na and NaCl levels, it may lead to a decline in yeast cell health and cell viability. In turn, this decrease may cause a delay in the onset and/or an increase in the duration of AF (Donkin *et al*., 2010). In addition, added osmotic pressure due to an increase in Na and Cl intake may cause harsh fermentation conditions for yeast cells which trigger an increase in acetic acid production (Tamas and Hohmann, 2003; Donkin *et al.,* 2010). An increase in acetic acid production in turn causes an increase in wine VA. Juice Na and Cl concentration was, however, not increased in this study (Table 4.4). Furthermore, wine NaCl levels of at least 33.59 g/L and 13.44 g/L are required to delay AF and promote VA production, respectively (Donkin *et al.,* 2010). The highest wine Na and Cl concentrations for all treatments in any of the two vintages were 32.8 mg/L and 56.4 mg/L, respectively (Table 4.4). Therefore, the levels were probably too low to influence fermentation performance and promote VA production. Still, care should be taken that high NaCl concentrations do not accumulate in juice and wine made from grapevines that receive Na-rich winery wastewater irrigation.

### 4.3.3 Wine composition

4.3.3.1 Standard wine analysis

The alcohol concentration and RS of bottled wines were not affected by the various wastewater irrigation treatments in either of the two vintages (Table 4.1). In addition, all wines fermented to dryness (< 5 g/L) indicating that none of the wastewater irrigation treatments had an effect on the ability of VIN 13 wine yeast to effectively complete AF. The various wastewater irrigation treatments did not affect residual glucose and fructose concentrations in any of the two vintages either. Therefore, the ability of the yeast strain to utilize these sugars was not affected. The amount of nitrogen ($NO_3^-$ + $NH_4^+$) applied to the soil *via* the different irrigation treatments as well as the amount of DAP added as yeast nutrition, were similar for all treatments. Therefore, as expected, final residual FAN in the bottled wines did not differ between any of the treatments in either of the vintages. There was no apparent seasonal variation with regards to any of the above mentioned parameters.

Wine TTA was not affected significantly by any of the wastewater irrigation treatments in either of the two vintages (Table 4.2). However, TTA for wines made from treatments that received irrigation with water containing high levels of COD tended to be higher than for wines made from treatments that received irrigation with low levels of COD in both seasons. The most important acid present in wine, namely tartaric acid, was not affected by irrigation using augmented winery wastewater in either of the vintages. The trend towards a decrease in wine TTA as the level of COD in the augmented winery wastewater increased can, therefore, not be attributed to a

decrease in tartaric acid. As all other forms of acid in wine were not quantified, the acid that was affected by the wastewater irrigation treatments could not be identified.

As mentioned earlier, malic acid concentrations of all wines were below 0.3 g/L, indicating that MLF was completed. Wine VA was not affected by any of the wastewater irrigation treatments in any of the two vintages. High levels of VA in wine generally cause a vinegar-like, pungent aroma in wine. Although VA values for all wines were relatively high in the 2011 vintage, close to the sensory detection threshold of 0.7 g/L, there were no trends with regards to treatments. In the 2012 vintage, VA concentrations for all wines were way below the detection threshold and once again no treatment trends were observed. Irrigation using augmented winery wastewater did, therefore, not cause an increase in wine VA. Several of the wastewater irrigation treatments increased wine pH when compared to the raw water irrigation control in the 2011 vintage. Raw water irrigation and wastewater irrigation treatments T2 and T3 produced wines with similar pH values, while wines from T4 tended towards a higher pH. Wastewater irrigation treatments 5, 6, 8 and 9 produced wines with higher pH values than wines made from T1, T2 and T3 and tended to have a higher pH than wines made from T4. Wines made from T7 had the highest pH value, higher than wines made from T1, T2, T3 and T4. In the 2012 vintage, wine pH was not affected statistically at a 95% confidence level. However, with the exception of T2, wine pH tended to increase as the level of COD in the irrigation water increased. The foregoing indicates a general trend towards increased wine pH with an increase in the level of COD in the augmented winery wastewater. Furthermore, the level of COD in augmented winery wastewater correlated closely with an increase in the amount of Na and K applied up to harvest through wastewater irrigation (Chapter 3, Figs. 3.2-3.5).

Therefore, the increase in wine pH and the trend towards a decrease in wine TTA as the level of COD in the augmented winery wastewater increased may be associated with the increased amounts of Na and K applied to soils with an increase in the level of COD in the augmented winery wastewater. Boulton (1980) reported measured wine pH to be a reflection of the extent to which protons from the total acidity were exchanged by K and Na ions. Furthermore, the uptake of K and Na at a constant total acidity can only lead to a rise in pH because the denominator of the extent of exchange term remains constant, while the numerator increases. Under these conditions the ratio of tartaric acid to malic acid remains unchanged, resulting in an increase in pH as the extent of exchange increases. He also reported that K concentration had a bigger effect on wine pH than juice pH. Other authors also found that excessive Na and K fertilization could cause salt formation from malic and tartaric acids, resulting in a decrease in TTA (Iland and Coombe, 1988; Mpelsoka *et al.,* 2003).

**Table 4.1** Results of chemical analysis of wines made from grapes of Cabernet Sauvignon/99R grapevines irrigated using augmented winery wastewater during the 2011 and 2012 vintages (each value represents the average of triplicate treatments).

| Treatment no. | Target COD (mg/L) | 2011 | | | | | 2012 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Alcohol (%v/v) | Reducing sugar (g/L) | Glucose (g/L) | Fructose (g/L) | Free amino nitrogen (mg/L) | Alcohol (%v/v) | Reducing sugar (g/L) | Glucose (g/L) | Fructose (g/L) | Free amino nitrogen (mg/L) |
| T1 | Raw water | 12.11 a[(1)] ± 0.39 | 1.89 a ± 0.68 | 0.06 a ± 0.06 | 0.07 a ± 0.03 | 116.7 a ± 3.2 | 12.65 a ± 0.61 | 1.66 a ± 0.15 | 0.08 a ± 0.04 | 0.07 a ± 0.01 | 119.5 a ± 1.6 |
| T2 | 100 | 11.91 a ± 0.64 | 1.25 a ± 0.15 | 0.06 a ± 0.04 | 0.06 a ± 0.04 | 122.3 a ± 8.6 | 11.93 a ± 0.22 | 1.70 a ± 0.52 | 0.05a ± 0.01 | 0.04 a ± 0.01 | 113.9 a ± 3.2 |
| T3 | 250 | 12.65 a ± 0.37 | 1.10 a ± 0. 02 | 0.05 a ± 0.06 | 0.08 a ± 0.01 | 112.9 a ± 1.6 | 11.96 a ± 0.28 | 1.76 a ± 0.14 | 0.06 a ± 0.03 | 0.03 a ± 0.01 | 115.7 a ± 3.2 |
| T4 | 500 | 12.36 a ± 0.38 | 1.96 a ± 0.14 | 0.04 a ± 0.05 | 0.06 a ± 0.05 | 121.3 a ± 4.3 | 12.34 a ± 0.65 | 1.61 a ± 0.18 | 0.07 a ± 0.01 | 0.06 a ± 0.01 | 118.5 a ± 4.3 |
| T5 | 1000 | 12.33 a ± 0.35 | 1.77 a ± 0.42 | 0.16 a ± 0.09 | 0.07 a ± 0.06 | 117.6 a ± 9.7 | 13.23 a ± 0.35 | 1.83 a ± 0.21 | 0.06 a ± 0.02 | 0.04 a ± 0.01 | 117.6 a ± 4.9 |
| T6 | 1500 | 12.80 a ± 0.40 | 1.22 a ± 0.21 | 0.09 a ± 0.05 | 0.09 a ± 0.02 | 112.0 a ± 0.1 | 12.19 a ± 0.23 | 1.58 a ± 0.08 | 0.07 a ± 0.02 | 0.05 a ± 0.01 | 116.7 a ± 3.2 |
| T7 | 2000 | 12.68 a ± 0.39 | 1.72 a ± 0.84 | 0.06 a ± 0.07 | 0.07 a ± 0.02 | 116.7a ± 5.8 | 12.13 a ± 0.22 | 1.41 a ± 0.11 | 0.07 a ± 0.01 | 0.04 a ± 0.01 | 115.7 a ± 1.6 |
| T8 | 2500 | 12.27 a ± 0.30 | 1.12 a ± 0.09 | 0.05 a ± 0.02 | 0.07 a ± 0.02 | 115.7 a ± 6.5 | 12.19 a ± 0.43 | 1.48 a ± 0.17 | 0.05 a ± 0.01 | 0.04 a ± 0.02 | 118.5 a ± 4.3 |
| T9 | 3000 | 13.00 a ± 0.10 | 1.29 a ± 0.27 | 0.05 a ± 0.03 | 0.08 a ± 0.03 | 114.8 a ± 2.8 | 13.00 a ± 0.64 | 1.94 a ± 0.21 | 0.06 a ± 0.03 | 0.06 a ± 0.01 | 115.7 a ± 3.2 |

[(1)] Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$)

± Values indicate standard deviation from the mean

**Table 4.2** Results of chemical analysis of wines made from grapes of Cabernet Sauvignon/99R grapevines irrigated using augmented winery wastewater during the 2011 and 2012 vintages (each value represents the average of triplicate treatments).

| Treatment no. | Target COD (mg/L) | 2010/11 | | | | | 2011/12 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | pH | Total titratable acidity (g/L) | Tartaric acid (g/L) | Malic acid (g/L) | Volatile acidity (g/L) | pH | Total titratable acidity (g/L) | Tartaric acid (g/L) | Malic acid (g/L) | Volatile acidity (g/L) |
| T1 | Raw water | 4.14 c[(1)] ± 0.11 | 4.01 a ± 0.37 | 1.20 a ± 0.13 | 0.01 a ± 0. 01 | 0.52 a ± 0.08 | 3.96 a ± 0.09 | 4.37 a ± 0.12 | 0.73 a ± 0.08 | 0.10 a ± 0.14 | 0.29 a ± 0.07 |
| T2 | 100 | 4.17 c ± 0.14 | 4.43 a ± 0.86 | 1.18 a ± 0.06 | 0.03 a ± 0.05 | 0.60 a ± 0.18 | 4.20 a ± 0.18 | 3.98 a ± 0.30 | 0.63 a ± 0.04 | 0.00 a ± 0.00 | 0.29 a ± 0.05 |
| T3 | 250 | 4.12 c ± 0.03 | 3.93 a ± 0.07 | 1.17 a ± 0.04 | 0.02 a ± 0.03 | 0.66 a ± 0.04 | 4.05 a ± 0.09 | 4.24 a ± 0.23 | 0.69 a ± 0.07 | 0.0 a ± 0.00 | 0.34 a ± 0.05 |
| T4 | 500 | 4.22 bc ± 0.11 | 4.21 a ± 0.34 | 1.24 a ± 0.05 | 0.14 a ± 0.08 | 0.70 a ± 0.10 | 4.06 a ± 0.15 | 4.26 a ± 0.34 | 0.70 a ± 0.09 | 0.25 a ± 0.38 | 0.31 a ± 0.08 |
| T5 | 1000 | 4.34 ab ± 0.09 | 3.71 a ± 0.23 | 1.28 a ± 0.03 | 0.00 a ± 0.00 | 0.61 a ± 0.10 | 4.09 a ± 0.02 | 4.26 a ± 0.15 | 0.67 a ± 0.04 | 0.05 a ± 0.08 | 0.31 a ± 0.03 |
| T6 | 1500 | 4.36 ab ± 0.06 | 3.63 a ± 0.13 | 1.30 a ± 0.02 | 0.02 a ± 0.04 | 0.55 a ± 0.11 | 4.16 a ± 0.09 | 4.15 a ± 0.28 | 0.81 a ± 0.22 | 0.21 a ± 0.26 | 0.37 a ± 0.03 |
| T7 | 2000 | 4.38 a ± 0.02 | 3.77 a ± 0.06 | 1.34 a ± 0.11 | 0.07 a ± 0.12 | 0.63 a ± 0.11 | 4.18 a ± 0.05 | 3.99 a ± 0.03 | 0.64 a ± 0.04 | 0.06 a ± 0.06 | 0.39 a ± 0.09 |
| T8 | 2500 | 4.33 ab ± 0.11 | 3.80 a ± 0.38 | 1.29 a ± 0.07 | 0.02 a ± 0.03 | 0.64 a ± 0.05 | 4.24 a ± 0.11 | 4.00 a ± 0.22 | 0.66 a ± 0.02 | 0.05 a ± 0.08 | 0.35 a ± 0.05 |
| T9 | 3000 | 4.34 ab ± 0.05 | 3.68 a ± 0.12 | 1.29 a ± 0.01 | 0.02 a ± 0.03 | 0.55 a ± 0.11 | 4.24 a ± 0.09 | 4.03 a ± 0.17 | 0.75 a ± 0.00 | 0.08 a ± 0.06 | 0.30 a ± 0.00 |

[(1)] Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$)

± Values indicate standard deviation from the mean

The generally high pH measured for all wines was a result of prolonged/delayed berry ripening due to a high frequency irrigation schedule. Grapes from Chenin blanc grapevines irrigated 28 days after véraison had a lower sugar concentration than grapevines irrigated only at pea size. Furthermore, grapevines that received irrigation 31 days after véraison, i.e. three days before harvest, produced berries with increased pH values when compared to grapevines receiving a single irrigation at pea size (Myburgh, 2006).

4.3.3.2 Colour, phenolics and tannin analyses

With the exception of T9 in the 2011 vintage, the various wastewater irrigation treatments had no effect on wine brown colour ($A_{420\ nm}$), when compared to the raw water irrigation control in either of the two vintages (Table 4.3). Although wines made from grapes of T9 had a higher brown hue in the 2011 vintage, this could not be related to the amount of brown colour pigments in berry skins. Furthermore, wine brown colour was similar for wines from all treatments in the 2012 vintage. There were no differences in the amount of red ($A_{520\ nm}$) colour pigments present in wines (Table 4.3). Even though wines made from T9 had the highest red colour hue in the 2011 vintage, it was not statistically higher than that of wines made from any of the other treatments and no differences were found in wine red colour hue in the 2012 vintage. The amount of red colour pigments in berry skins was also similar for all treatments. Wine colour density was similar for all wines in both vintages and, therefore, not affected by the various wastewater irrigation treatments. Wines made from all treatments had a light red colour ($A_{420\ nm} + A_{520\ nm} \leq 6$) in both vintages, probably because grapevines received adequate irrigation throughout the season or due to the rapid completion of AF which limited the time available for extraction from berry skins. Grapevines receiving adequate irrigation produce lower anthocyanins per gram of berry skin when compared to grapevines receiving strong water constraints (Ojeda *et al.*, 2002). A considerable decrease in wine brown and red colour, as well as colour density was observed from the 2011 to the 2012 vintage. This decrease was, however, noticed for wines made from all treatments and could, thus, not be related to a wastewater irrigation treatment effect. Similar to these results, the degree of red pigment colouring was not affected by the various irrigation treatments and also decreased largely from the 2011 to the 2012 vintage. There were no differences in the total amount of phenols present in wines made from any of the treatments in either of the vintages. As berry skin total phenolics were not affected, the similarity in wine total phenolics was to be expected. Furthermore, the wine total phenolic content did not vary between the two vintages. Wine tannin content was similar for all treatments during both vintages and remained mostly unchanged in concentration from the 2011 to the 2012 vintage. The similarities in wine colour, phenolic and tannin composition indicate that the ability of the grapevine berries to accumulate these compounds, their extraction in the wine medium and the changes which occur to these compounds during vinification was not affected by irrigation using augmented winery wastewater.

**Table 4.3** Results of chemical analysis of wines made from grapes of Cabernet Sauvignon/99R grapevines irrigated using augmented winery wastewater during the 2011 and 2012 vintages (each value represents the average of triplicate treatments).

| Treatment no. | Target COD (mg/L) | 2011 | | | | | | 2012 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Colour ($A_{420\ nm}$) | Colour ($A_{520\ nm}$) | Colour density ($A_{420\ nm + 520\ nm}$) | Degree of red pigment colouring (%) | Total phenols ($A_{280\ nm}$) | Total Tannins (g/L) | Colour ($A_{420\ nm}$) | Colour ($A_{520\ nm}$) | Colour density ($A_{420\ nm + 520\ nm}$) | Degree of red pigment colouring (%) | Total phenols ($A_{280\ nm}$) | Total Tannins (g/L) |
| T1 | Raw water | 2.01 bc[(1)]* ± 0.3 | 1.92 a* ± 0.5 | 3.93 a* ± 0.7 | 17.44 a ± 2.3 | 34.44 a* ± 3.4 | 0.82 a ± 0.3 | 0.34 a* ± 0.1 | 0.45 a* ± 0.3 | 0.79 a* ± 0.4 | 4.00 a ± 2.0 | 36.10 a* ± 1.5 | 0.47 a ± 0.3 |
| T2 | 100 | 1.76 c ± 0.1 | 1.68 a ± 0.3 | 3.43 a ± 0.3 | 17.87 a ± 1.3 | 33.67 a ± 0.7 | 0.47 a ± 0.2 | 0.26 a ± 0.0 | 0.28 a ± 0.1 | 0.54 a ± 0.1 | 2.69 a ± 1.1 | 31.79 a ± 0.4 | 0.81 a ± 0.4 |
| T3 | 250 | 1.95 bc ± 0.1 | 1.79 a ± 0.2 | 3.74 a ± 0.3 | 18.48 a ± 2.2 | 34.68 a ± 1.2 | 0.61 a ± 0.1 | 0.27 a ± 0.1 | 0.38 a ± 0.1 | 0.65 a ± 0.2 | 3.19 a ± 1.0 | 34.62 a ± 1.8 | 0.86 a ± 0.1 |
| T4 | 500 | 1.94 bc ± 0.1 | 1.74 a ± 0.1 | 3.68 a ± 0.1 | 15.94 a ± 1.7 | 36.80 a ± 1.8 | 1.10 a ± 0.4 | 0.35 a ± 0.2 | 0.47 a ± 0.3 | 0.82 a ± 0.5 | 4.55 a ± 2.1 | 34.62 a ± 4.1 | 0.67 a ± 0.2 |
| T5 | 1000 | 2.22 ab ± 0.2 | 1.98 a ± 0.2 | 4.2 a ± 0.3 | 17.45 a ± 1.8 | 34.75 a ± 2.0 | 1.09 a ± 0.6 | 0.50 a ± 0.1 | 0.53 a ± 0.1 | 1.04 a ± 0.1 | 4.90 a ± 0.3 | 36.33 a ± 3.0 | 1.00 a ± 0.3 |
| T6 | 1500 | 2.07 bc ± 0.1 | 1.71 a ± 0.2 | 3.78 a ± 0.0 | 14.34 a ± 1.8 | 34.72 a ± 1.0 | 0.84 a ± 0.2 | 0.32 a ± 0.1 | 0.38 a ± 0.1 | 0.47 a ± 0.4 | 3.14 a ± 2.2 | 34.68 a ± 2.0 | 0.72 a ± 0.5 |
| T7 | 2000 | 2.25 ab ± 0.3 | 2.14 a ± 0.4 | 4.38 a ± 0.8 | 19.05 a ± 3.8 | 36.91 a ± 3.5 | 1.13 a ± 0.2 | 0.26 a ± 0.1 | 0.30 a ± 0.1 | 0.56 a ± 0.2 | 3.25 a ± 0.9 | 35.52 a ± 1.0 | 0.94 a ± 0.1 |
| T8 | 2500 | 2.00 bc ± 0.2 | 1.79 a ± 0.2 | 3.79 a ± 0.4 | 16.29 a ± 0.9 | 33.98 a ± 1.8 | 1.39 a ± 0.7 | 0.28 a ± 0.0 | 0.24 a ± 0.1 | 0.52 a ± 0.1 | 2.25 a ± 0.7 | 33.91 a ± 2.6 | 0.92 a ± 0.1 |
| T9 | 3000 | 2.53 a ± 0.3 | 2.45 a ± 0.6 | 4.98 a ± 0.9 | 22.39 a ± 12.2 | 36.84 a ± 2.8 | 1.02 a ± 0.1 | 0.37 a ± 0.2 | 0.45 a ± 0.3 | 0.81 a ± 0.5 | 4.39 a ± 2.5 | 35.42 a ± 1.3 | 0.51 a ± 0.4 |

[(1)] Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$)

*Values refer to absorbance units

± Values indicate standard deviation from the mean

With an increase in wine pH, the anthocyanin colour equilibrium shifts towards different colour forms (Ribéreau-Gayon *et al.,* 2006). An increase in wine pH causes some of the red coloured flavilium cations to be converted into carbinol bases (colourless), cis- and trans-chalcone (yellow) and quinoidal base (blue), thus, causing a decline in wine red colour (Ribéreau-Gayon *et al.,* 2006). A reduction in wine red colour hue and colour density, related to an increase in wine pH, was not observed in this study, probably because the increases in wine pH as the level of COD in the augmented winery wastewater increased was not large enough.

4.3.3.3 Phosphorus and selected ion composition

The various wastewater irrigation treatments had no effect on wine P and selected ion (Na, K, Cl) contents in wine in either the 2011 or the 2012 vintage (Table 4.4). Therefore, even though P, Na and K were applied in increasing amounts as the level of COD in the augmented winery wastewater increased, they were not elevated in the wines made from any of the treatments (Table 3.1). Even though juice Na was increased as the level of COD in the augmented winery wastewater increased in the 2011 vintage, this increase was not observed in the wine Na concentration. The similarities in the concentration of these compounds in the wines suggest that P and these ions were not absorbed to a larger extent as larger amounts were applied through irrigation. There are currently no definite values which restrict the P and K concentration in wines. The must P levels were consistent with levels obtained from other studies (Conradie, 2001; Bruwer, 2010). Levels of K in wines also coincided with results obtained by Berg *et al*. (1979). Sodium concentration for all wines was comfortably within the maximum limit of 100 mg/L for South African wines (Department of Water Affairs and Forestry, 1996). Wine P, Na and K concentration for all wines decreased from the 2011 to the 2012 vintage, therefore indicating that P, Na and K probably did not accumulate in the soil from the 2011 to the 2012 season or was not absorbed by the grapevine to a greater extent in the 2012 season.

As the amount of Cl applied *via* irrigation water did not increase as the level of COD in the irrigation water increased, Cl concentration in the wine was not affected by any of the wastewater irrigation treatments in any of the two vintages. Wine Cl concentration did however increase from the 2011 to the 2012 vintage for all treatments. If soil Cl accumulates with seasons and is absorbed to a larger extent by grapevines, it may lead to toxic effects on grapevine growth and an increase in wine Cl content. However, the wine Cl content was within reported norms for juice Cl content and far below the maximum wine Cl level of 606 mg/L for Australian wines (Leske *et al.,* 1997). Furthermore, the maximum Cl level for all irrigations during any time of the two seasons was 60.5 mg/L. This is far below the recommended concentration of less than 100 mg/L, with a caution that levels between 70 mg/L and 175 mg/L can be harmful (Van Zyl, 1981; Department of Water Affairs and Forestry, 1996; ANZECC, 2000). Therefore, irrigation using augmented winery wastewater should not hold a risk for Cl

toxicity. Still, it is important to measure Cl content of water which will be used for the purpose of irrigation.

The $SO_4^{2-}$ concentration in the wines was not affected by the various wastewater irrigation treatments in the 2011 vintage. The 2012 vintage showed considerable variability between the different treatments. Still, no definite trend was observed with regards to the irrigation treatments. In addition, no definite trend was observed in the amount of $SO_4^{2-}$ applied through the various irrigation water treatments either, explaining the lack of trends in wine $SO_4^{2-}$ content. The amount of $SO_4^{2-}$ in wines decreased largely from the 2011 to the 2012 vintage. The amount of $SO_4^{2-}$ applied through irrigation was also higher in the 2011 season. Furthermore, the highest wine $SO_4^{2-}$ levels, in the 2012 vintage, were found in wines made from T5, T7 and T9. These were also the treatments where the highest amount of $SO_4^{2-}$ was applied through the irrigation water, thus, indicating that $SO_4^{2-}$ may be absorbed to a larger extent as the amount applied through irrigation water increases. Maximum allowed potassium sulphate levels for South African and German wines are 2000 mg/L and 1000 mg/L, respectively. Wines made from all treatments had $SO_4^{2-}$ levels comfortably below this level.

Elements such as Na, K, Mg and Ca are usually present at much higher concentrations in grape berry skins than in pulp (Son *et al.,* 2009). Therefore, enhanced extraction when conducting AF on the skins, due to an increase in temperature and the production of alcohol, should result in higher concentrations of these elements in wines than in juice. In the 2011 vintage, P, Na and K levels increase considerably from the juice to wine, probably due to increased extraction as mentioned above. In the 2012 vintage, however, P, Na and K concentration decreased from juice to wine, thus indicating that P, Na and K were either deposited as sediment or bound to other compounds. Similar increases and decreases in elements from grape juice to wine have been reported by other authors (Garcia *et al.,* 2001; Bruwer, 2010), indicating that elements are not necessarily extracted in large amounts during AF or that the elements are removed from the wine in some way or another.

**Table 4.4** Phosphorus and selected cation and anion concentrations in wines made from grapes of Cabernet Sauvignon/99R grapevines irrigated using augmented winery wastewater during the 2011 and 2012 vintages (each value represents the average of triplicate treatments).

| Treatment no. | Target COD (mg/L) | 2010/11 | | | | | 2011/12 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | P (mg/L) | Na (mg/L) | K (mg/L) | Cl (mg/L) | $SO_4^{2-}$ (mg/L) | P (mg/L) | Na (mg/L) | K (mg/L) | Cl (mg/L) | $SO_4^{2-}$ (mg/L) |
| T1 | Raw water | 412.4 a[(1)] ± 13.9 | 32.8 a ± 0.9 | 1740.3 a ± 268 | 35.5 a ± 8.9 | 587.5 a ± 53.7 | 175.0 a ± 24.7 | 21.6 a ± 3.8 | 981.5 a ± 145 | 38.6 a ± 14.5 | 115.9 bcd ± 14.5 |
| T2 | 100 | 423.4 a ± 31.0 | 31.7 a ± 0.8 | 1971.1 a ± 390 | 41.4 a ± 13.6 | 542.5 a ± 30.6 | 184.3 a ± 6.9 | 18.7 a ± 0.9 | 1008.0 a ± 210 | 47.5 a ± 5.1 | 99.0 cd ± 0.7 |
| T3 | 250 | 418.6 a ± 8.6 | 28.9 a ± 2.0 | 1661.6 a ± 126 | 26.6 a ± 8.9 | 537.6 a ± 26.1 | 170.0 a ± 9.1 | 17.6 a ± 0.7 | 844.8 a ± 101 | 47.5 a ± 18.5 | 95.7 d ± 24.0 |
| T4 | 500 | 411.3 a ± 14.3 | 30.4 a ± 2.1 | 1708.6 a ± 242 | 35.5 a ± 8.9 | 565.3 a ± 26.6 | 172.9 a ± 8.3 | 17.5 a ± 1.3 | 836.1 a ± 95 | 44.5 a ± 15.4 | 108.5 bcd ± 3.6 |
| T5 | 1000 | 384.4 a ± 26.3 | 29.9 a ± 1.5 | 1826.3 a ± 210 | 23.7 a ± 10.3 | 556.1 a ± 33.8 | 183.4 a ± 5.8 | 19.7 a ± 0.6 | 948.9 a ± 68 | 44.5 a ± 0.0 | 130.7 abc ± 16.9 |
| T6 | 1500 | 373.9 a ± 8.0 | 28.9 a ± 3.8 | 1879.0 a ± 139 | 35.5 a ± 8.9 | 548.7 a ± 9.2 | 166.8 a ± 3.5 | 18.7 a ± 1.2 | 926.6 a ± 51 | 38.6 a ± 10.3 | 108.1 bcd ± 33.7 |
| T7 | 2000 | 411.2 a ± 13.0 | 28.0 a ± 1.4 | 2023.3 a ± 20 | 38.5 a ± 13.6 | 550.3 a ± 24.3 | 171.0 a ± 6.8 | 17.6 a ± 0.9 | 952.7 a ± 46 | 38.6 a ± 10.3 | 130.8 abc ± 9.2 |
| T8 | 2500 | 402.7 a ± 24.3 | 28.6 a ± 2.5 | 1932.7 a ± 247 | 32.6 a ± 5.1 | 565.5 a ± 6.6 | 168.1 a ± 7.1 | 18.2 a ± 1.7 | 986.9 a ± 173 | 44.5 a ± 8.9 | 136.9 ab ± 15.4 |
| T9 | 3000 | 405.4 a ± 7.2 | 31.8 a ± 3.0 | 1929.2 a ± 106 | 35.5 a ± 17.8 | 547.1 a ± 12.2 | 183.5 a ± 7.9 | 19.1 a ± 0.3 | 1078.5 a ± 53 | 56.4 a ± 10.3 | 152.8 a ± 25.3 |

[(1)] Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$)

± Values indicate standard deviation from the mean

### 4.3.4 Wine sensorial characteristics

Visually, wines were consistent with wet chemistry analysis, indicating that wine colour was not affected by any of the wastewater irrigation treatments in either of the vintages (Table 4.5).

Wine sensorial evaluation indicated no differences between wines with regards to overall flavour intensity, vegetative character, berry character and spicy character. All wines tended to have a stronger berry-like character than vegetative or spicy characters, consistent with Cabernet Sauvignon wine made from ripe berries from warmer localities such as Rawsonville. All the above mentioned sensory descriptive parameters were scored low marks, indicating a lack of wine flavour intensity which could be explained by the adequate amount of irrigation received throughout the seasons. Chapman *et al.* (2005) reported wines made from Cabernet Sauvignon vines that received minimal irrigation to be rated higher in all the fruity descriptors (fresh cherry, black berry, cooked berry and dried fruit), when compared to wines made from standard irrigated and double standard irrigated grapevines. Other authors also reported that grapevines which received high frequency irrigation during ripening produced wines with diluted character flavours and aroma, resulting in an inferior overall quality (Lategan, 2011; Myburgh, 2011).

No differences were found between any of the treatments when comparing off-odour and off-taste intensity. Both, off-odours and off-tastes, were scored very low for all treatments in both seasons, the highest being 16.7/100 and 11.5/100, respectively. The low scores for these two parameters in wines made from all treatments indicated that no wastewater associated off-odours or off-tastes were transferred into wines or produced as a response to wastewater irrigation. The off-odours and off-tastes that were observed were all related to frequently occurring off-odours and off-tastes in wines, VA and bitterness being the most prominent. When applying the various irrigation treatments, a prominent foul wastewater-like odour was noticed in grapevine plots that received irrigation treatments containing >2000 mg/L COD. It has previously been shown that certain odours may be transferred from the surrounding environment into grapes and the resulting wines. A Study by Kennison *et al.* (2009) reported that, when grapevines were exposed to smoke between véraison and harvest it caused a ‘smoke taint’ in the resulting wines. Similarly, wines made from grapevines which is situated nearby *Eucalyptus* tree plantations, has been found to obtain higher *Eucalyptus*-like or minty characters which may be obtained from the trees (Novak, 2002; Van Leeuwen *et al*., 2007).

Wine acidity and body were not influenced by the wastewater irrigation treatments in either of the seasons, consistent with wet chemistry analysis. Even though wine TTA tended to decrease as the level of COD in the augmented winery wastewater increased, differences were probably too small to notice during sensorial evaluation.

**Table 4.5** Results of sensory evaluation of wines made from grapes of Cabernet Sauvignon/99R grapevines irrigated using augmented winery wastewater during the 2011 and 2012 vintages (each value represents the average of triplicate treatments).

| Treatment no. | Target COD (mg/L) | Colour | Overall intensity | Vegetative | Berry | Spicy | Off odours | Acidity | Fullness | Astringency | Off tastes | Overall quality |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **2010/11** | | | | | | | | | | |
| T1 | Raw water | 44.9 a[1, 2] ± 13.5 | 48.3 a ± 7.0 | 31.0 a ± 13.4 | 40.6 a ± 8.5 | 21.8 a ± 0.7 | 8.1 a ± 4.9 | 37.0 a ± 3.8 | 37.6 a ± 6.6 | 24.7 a ± 1.2 | 7.5 a ± 2.4 | 43.4 a ± 8.3 |
| T2 | 100 | 32.2 a ± 16.2 | 46.9 a ± 4.0 | 25.0 a ± 2.6 | 30.2 a ± 3.6 | 12.4 a ± 1.1 | 16.7 a ± 4.6 | 41.4 a ± 6.7 | 31.9 a ± 7.2 | 25.3 a ± 0.3 | 10.8 a ± 3.2 | 33.2 a ± 5.7 |
| T3 | 250 | 44.4 a ± 9.0 | 44.4 a ± 5.1 | 21.7 a ± 5.3 | 40.2 a ± 2.9 | 18.5 a ± 4.7 | 4.8 a ± 3.6 | 39.0 a ± 0.8 | 36.1 a ± 3.7 | 29.4 a ± 2.3 | 9.0 a ± 5.1 | 38.4 a ± 8.8 |
| T4 | 500 | 43.1 a ± 6.5 | 48.4 a ± 2.6 | 24.4 a ± 2.3 | 42.8 a ± 2.2 | 22.8 a ± 5.6 | 7.1 a ± 5.3 | 38.8 a ± 0.7 | 37.3 a ± 3.4 | 28.7 a ± 4.1 | 8.4 a ± 2.3 | 41.2 a ± 4.5 |
| T5 | 1000 | 53.9 a ± 3.3 | 50.7 a ± 1.4 | 29.3 a ± 6.5 | 45.9 a ± 6.6 | 19.8 a ± 3.2 | 4.5 a ± 2.9 | 38.3 a ± 3.6 | 41.1 a ± 2.6 | 26.6 a ± 1.2 | 5.2 a ± 2.0 | 43.8 a ± 2.6 |
| T6 | 1500 | 36.0 a ± 7.1 | 40.4 a ± 7.2 | 31.4 a ± 8.1 | 30.8 a ± 5.7 | 14.6 a ± 4.7 | 4.7 a ± 1.3 | 37.9 a ± 1.4 | 31.8 a ± 3.4 | 23.9 a ± 2.8 | 5.2 a ± 5.1 | 32.4 a ± 3.3 |
| T7 | 2000 | 55.0 a ± 11.5 | 47.6 a ± 6.2 | 32.3 a ± 0.8 | 37.3 a ± 9.0 | 18.3 a ± 5.0 | 4.5 a ± 4.4 | 43.0 a ± 2.7 | 38.7 a ± 5.1 | 28.8 a ± 5.0 | 6.0 a ± 3.3 | 43.5 a ± 4.5 |
| T8 | 2500 | 40.9 a ± 14.5 | 45.8 a ± 5.5 | 29.3 a ± 5.7 | 34.3 a ± 10.9 | 17.7 a ± 5.7 | 11.8 a ± 3.1 | 40.9 a ± 4.4 | 36.5 a ± 5.2 | 27.0 a ± 3.4 | 8.7 a ± 3.5 | 38.0 a ± 6.4 |
| T9 | 3000 | 53.9 a ± 16.5 | 52.0 a ± 5.9 | 25.1 a ± 5.2 | 45.8 a ± 8.7 | 17.7 a ± 0.9 | 12.8 a ± 7.9 | 40.5 a ± 1.4 | 40.8 a ± 5.0 | 30.5 a ± 0.6 | 11.5 a ± 13.6 | 44.1 a ± 3.5 |
| | | **2011/12** | | | | | | | | | | |
| T1 | Raw water | 32.9 a ± 2.4 | 50.9 a ± 5.0 | 25.6 a ± 0.5 | 39.2 a ± 6.3 | 17.0 a ± 4.6 | 5.9 a ± 1.6 | 33.9 a ± 3.1 | 34.8 a ± 2.5 | 33.9 a ± 5.4 | 9.9 a ± 3.1 | 37.8 a ± 2.3 |
| T2 | 100 | 29.8 a ± 0.8 | 44.9 a ± 1.4 | 23.3 a ± 5.8 | 31.5 a ± 0.7 | 17.0 a ± 1.7 | 7.1 a ± 2.1 | 31.4 a ± 1.6 | 31.6 a ± 1.1 | 28.9 a ± 2.8 | 8.5 a ± 0.7 | 34.7 a ± 0.9 |
| T3 | 250 | 32.4 a ± 2.9 | 45.9 a ± 5.9 | 23.8 a ± 3.7 | 34.3 a ± 5.1 | 17.5 a ± 3.3 | 5.1 a ± 0.8 | 33.7 a ± 2.3 | 35.2 a ± 2.2 | 32.5 a ± 3.3 | 9.5 a ± 4.9 | 39.0 a ± 1.8 |
| T4 | 500 | 33.1 a ± 7.8 | 47.9 a ± 5.5 | 26.7 a ± 0.2 | 34.9 a ± 8.5 | 17.1 a ± 4.1 | 6.4 a ± 2.4 | 33.4 a ± 4.9 | 32.8 a ± 4.0 | 27.3 a ± 2.2 | 9.6 a ± 2.6 | 34.6 a ± 4.0 |
| T5 | 1000 | 38.7 a ± 3.7 | 50.9 a ± 0.2 | 22.9 a ± 3.8 | 41.6 a ± 4.6 | 18.1 a ± 1.5 | 4.4 a ± 1.5 | 34.8 a ± 4.7 | 37.5 a ± 1.8 | 30.4 a ± 2.7 | 8.8 a ± 4.0 | 39.3 a ± 2.4 |
| T6 | 1500 | 33.8 a ± 3.0 | 47.4 a ± 4.7 | 18.8 a ± 1.1 | 38.0 a ± 4.7 | 13.5 a ± 2.5 | 4.0 a ± 0.4 | 31.5 a ± 3.4 | 31.5 a ± 1.3 | 26.8 a ± 2.3 | 7.0 a ± 3.0 | 37.0 a ± 0.8 |
| T7 | 2000 | 34.2 a ± 5.5 | 42.5 a ± 4.1 | 19.5 a ± 1.6 | 31.4 a ± 4.0 | 13.6 a ± 1.1 | 3.9 a ± 1.5 | 30.6 a ± 2.5 | 32.1 a ± 3.5 | 26.7 a ± 1.9 | 6.6 a ± 2.7 | 36.0 a ± 2.5 |
| T8 | 2500 | 31.8 a ± 5.1 | 46.6 a ± 1.7 | 21.3 a ± 0.6 | 37.2 a ± 3.2 | 14.9 a ± 4.1 | 4.1 a ± 0.7 | 31.4 a ± 2.6 | 33.0 a ± 4.0 | 27.5 a ± 1.7 | 7.0 a ± 1.8 | 37.0 a ± 5.3 |
| T9 | 3000 | 40.0 a ± 8.0 | 48.5 a ± 2.1 | 24.6 a ± 4.1 | 39.0 a ± 4.3 | 16.9 a ± 4.9 | 2.9 a ± 0.9 | 33.3 a ± 4.3 | 35.2 a ± 2.6 | 31.6 a ± 2.0 | 4.6 a ± 2.1 | 37.7 a ± 4.1 |

[1] Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$)
[2] Values represent a score out of 100
± Values indicate standard deviation from the mean

Overall wine quality was not affected by the various wastewater irrigation treatments as suggested by the absence of treatment effects for the other sensory parameters. For the most part, sensorial wine descriptors were scored slightly lower in the 2012 vintage than in the 2011 vintage. The lower scores could be attributed to wines being even lighter/more diluted than in the 2011 season. Strangely, berry skin colour and phenolics were higher in the 2011/12 season and would have been expected to lead to the production of fuller wines with better colour. The extraction of colour and phenolic compounds was probably better in the 2010/11 season, resulting in fuller wines with better colour.

In general, irrigation using augmented winery wastewater did not affect sensorial wine quality. This further establishes other findings in this study which indicates that, with the exception of TTA and pH, wastewater irrigation did not alter any of the wine quality determining factors significantly. High pH wines generally taste flat and may result in red wines with a brownish hue (Gladstones 1992; Rühl 1989). However, pH increases observed in this study were probably not substantial enough to cause a noticeable effect on sensory wine characteristics.

## 4.4 CONCLUSIONS

Irrigation with augmented winery wastewater within the applied ranges did not seem to affect the natural yeast and bacteria flora of grape must. This indicates that yeast and bacterial cells are probably not transferred into the grape must as a result of wastewater irrigation. Variation in must microbial flora could be ascribed to a variation in naturally occurring climatic and cultivation conditions as well as microflora within the vineyard, rather than to a treatment effect. Furthermore, as sufficient *S. cerevisiae* and *O. oeni* cell numbers are retained in the fermentations, AF and MLF are completed without any problems. Therefore, sugar and malic acid utilization is not affected by wastewater irrigation. These findings indicate that grape must and wine composition is not altered by irrigation using augmented winery wastewater in such a manner that microbial growth and survival or their ability to ferment was affected negatively.

Total titratable acidity of bottled wines tend to be higher for wines made from grapevines which receive irrigation with water containing higher levels of COD. Related to a decrease in TTA, wine pH tends to increase. Furthermore, the tempo of MLF may be faster for higher pH wines due to an increase in malolactic activity at these higher pH values. This increase in pH and malolactic activity may be especially important for cooler wine producing regions that generally produce lower pH wines. Furthermore, the TTA and pH effect, which would be mostly negative for South African wines, can be rectified by simply adding acid during the winemaking process. Irrigation using augmented winery wastewater did not alter the must and wine matrix in such a manner that VA production by yeast and LAB species was stimulated.

Wine red and brown colour hue, degree of red pigment colouring, total phenolics and tannins were not affected by wastewater irrigation under the given circumstances. Therefore, their synthesis and accumulation in berries, extraction in the wine medium, and changes to these compounds during vinification is not affected by irrigation using augmented winery wastewater. Even though increases in wine pH have been known to affect wine colour negatively, it was not observed in this study.

Wine P, Na, K, Cl and $SO_4^{2-}$ concentration was not affected by grapevine irrigation with augmented winery wastewater, even though P, Na and K were applied in greater amounts as the level of COD in the augmented winery wastewater increased. These findings indicated that these elements were not absorbed into grapes in larger amounts, as larger quantities were applied *via* irrigation water. Another possibility may be that these elements were absorbed in increasing amounts, but were not extracted during winemaking or they were bound to other compounds in the wine and not measured during analysis. Furthermore, as P, Na and K were present at lower levels in the 2012 vintage, they probably did not build up in the soil from the 2010/11 to the 2011/12 season.

Wine sensorial evaluation clearly showed that irrigation using augmented winery wastewater does not alter sensorial wine attributes or quality. Wines from all treatments were rather thin, lacking colour and flavour, due to the adequate irrigation it received throughout the season and the rapid completion of AF. Furthermore, as none of the wines contained off-flavours, it may be assumed that off-flavours are not transferred from wastewater into the wines and that juice and wine composition is not altered in such a manner that off-flavour production by microbes is stimulated.

Yeast and bacteria cells were not transferred from the wastewater irrigation treatments into the juice and wine. Therefore, any pathogenic organisms that might be contained within the wastewater should not be transferred either, allowing the conclusion that vineyard irrigation with winery wastewater does not cause the resulting wines to be unsafe for consumer consumption.

According to findings in this study it does seem like irrigation using augmented winery wastewater may be considered a viable option under the given conditions. However, it is important to remember that these results were obtained as a two year response. Effects may be accumulative and only start to negatively impact on soil, grapevine and wine quality after several years of application. Furthermore, it is possible that entirely different or greater effects may be observed when augmented winery wastewater is applied over a longer period, under different field and climatic conditions, from wastewater generated from a different cellar.

Applying wastewater irrigation to heavier soils, for instance, may lead to much more rapid and severe effects. Irrigation using more concentrated winery wastewater or that with a different composition may also have a greater effect on grapevine performance as well as juice and wine quality.

## 4.5 LITERATURE CITED


ANZECC, 2000. Australian and New Zealand guidelines for fresh and marine water quality: Volume 3 - primary industries - rationale and background information. Chapter 9. (http://www.environment.gov.au/water/publications/quality/pubs/nwqms-guidelines-4-vol3.pdf).

Bartowsky, E.J. & Henschke, P.A., 1995. Malolactic fermentation and wine flavour. Aust. Grapegrow. Winemak. 378, 83-94.

Bauer, R. & Dicks, L.M.T., 2004. Control of malolactic fermentation in wine. A review. S. Afr. J. Enol. Vitic. 25, 74-88.

Boulton, R., 1980. The general relationship between potassium, sodium and pH in grape juice and wine. Am. J. Enol. Vitic. 31, 182-186.

Bousbouras, G.E. & Kunkee, R.E., 1971. Effect of pH on malo-lactic fermentation in wine. Am. J. Enol. Vitic. 22, 121-126.

Bruwer, R.J., 2010. The edaphic and climatic effects on production and wine quality of Cabernet Sauvignon in the Lower Olifants River region. MSc thesis, University of Stellenbosch, Private bag X1, 7602 Matieland (Stellenbosch), South Africa.

Buchanan, R.E. & Gibbons, N.E., 1974. Bergey's manual of determinative bacteriology (8$^{th}$ ed). The Williams & Wilkins Company, Baltimore.

Chapman, J.A., 1996. Cleaner production for the wine industry. South Australian Wine and Brandy Industry Association, Adelaide, Australia.

Chapman, D.M., Roby, G., Ebeler, S.E., Guinard, J. & Matthews, M.A., 2005. Sensory attributes of Cabernet Sauvignon wines made from vines with different water status. Aust. J. Grape Wine Res. 11, 339-347.

Clesceri, L.S., Greenberg, A.E. & Eaton, A.D., 1998 (20$^{th}$ ed). Standard methods for the examination of water and waste water. Am. J. Public Health 4, 117-122.

Conradie, W.J., 2001. Timing of nitrogen fertilisation and the effect of poultry manure on the performance of grapevines on sandy soil. II. Leaf analysis, juice analysis and wine quality. S. Afr. J. Enol. Vitic. 22, 60-68.

Davis, C.R., Wibowo, D., Eschenbruch, R., Lee, T.H. & Fleet, G.H., 1985. Practical implications of malolactic fermentation: A review. Am. J. Enol. Vitic. 36, 290-301.

Davis, CR., Wibowo, D., Fleet, GH., Lee, TH., 1988. Properties of wine lactic acid bacteria: their potential enological significance. Am. J. Enol. Vitic. 39, 137-142.

Department of Environmental Affairs and Tourism (DEAT), 2004. A national climate change response strategy for South Africa. (unfccc.int/files/meetings/seminar/.../pdf/sem_sup3_south_africa.pdf).

Department of Water Affairs and Forestry (DWAF), 1996. South African water quality guidelines. Vol. 4, Agricultural use: irrigation. CSIR Environmental Services. Department of Water Affairs and Forestry, Pretoria.

Di Maro, E., Ercolini, D. & Coppola, S., 2007. Yeast dynamics during spontaneous wine fermentation of the Catalanesca grape. Int. J. Food. Microbiol. 117, 201-210.

Donkin, R., Robinson, S., Sumby, K., Harris, V., McBryde, C. & Jiranek, V., 2010. Sodium chloride in Australian grape juice and its effect on alcoholic and malolactic fermentation. Am. J. Enol. Vitic. 61, 392-400.

Drici-Cachon, A., Guzzo, J., Cavin, F. & Diviès, C., 1996. Acid tolerance in *Leuconostoc oenos*. Isolation and characterisation of an acid resistant mutant. Appl. Microbiol. Biotech. 44, 785-789.

Fleet, G.H. & Heard, G.M., 1993. Yeast – growth during fermentation. In: Fleet, G.H. (ed). Wine microbiology and biotechnology, Hardwood Academic Publishers, Singapore. pp. 27-54.

Foss Analytical, Denmark. (http:www.foss.dk).

Garcia, M., Ibrahim, H., Gallego, P. & Puig, P., 2001. Effect of three rootstocks on grapevine (*Vitis Vinifera* L.) cv. Négrette, grown hydroponically. II. Acidity of musts and wines. S. Afr. J. Enol. Vitic. 22, 104-106.

Gladstones, J., 1992. Viticulture and environment: A study of the effects of environment on grape-growing and wine qualities, with emphasis on present and future areas for growing winegrapes in Australia. Winetitles, Adelaide.

Henick-Kling, T., 1993. Malolactic fermentation. In: Fleet, G.H. (ed.). Wine Microbiology and Biotechnology, Harwood Academic Publishers, Chur, Switzerland, pp. 289-326.

Henick-Kling, T. & Park, Y.H., 1994. Considerations for the use of yeast and bacterial starter cultures: $SO_2$ and timing of inoculation. Am. J. Enol. Vitic. 45, 464-469.

Iland, P.G. & Coombe, B.G., 1988. Malate, tartrate, potassium and sodium in flesh and skin of Shiraz grapes during ripening: concentration and compartementation. Am. J. Enol. Vitic. 39, 71-76.

Iland, P., Ewart, A., Sitters, J., Markides, A. & Bruer, N., 2000. Techniques for chemical analysis and quality monitoring during winemaking. Patrick Iland Wine Promotions Pty Ltd. Campbelltown, Australia.

Irwin, O.R., Subden, R., Lautensach, A. & Cunningham, J.P., 1983. Genetic heterogeneity in lactobacilli and leuconostocs of enological significance. Can. Inst. Food Sci. Technol. J. 16, 79-81.

Jolly, N.P., Augustyn, O.P.H. & Pretorius, I.S., 2003. The occurrence of Non-*Saccharomyces cerevisiae* yeast species over three vintages in four vineyards and grape must from four production regions of the Western Cape, South Africa. S. Afr. J. Enol. Vitic. 24, 35-42.

Jourjon, F., Khaldi, S., Reveillere, M., Thibault, C., Poulard, A., Chretien, P. & Bednar, J., 2005. Microbiological characterization of winery effluent: an inventory of the sites for different treatment systems. Water Sci. Technol. 51, 19-26.

Kennison, K.R., Wilkinson, K.L., Pollnitz, A.P., Williams, H.G. & Gibberd, M.R., 2009. Effect of timing and duration of grapevine exposure to smoke on the composition and sensory properties of wine. Aust. J. Grape Wine Res. 15, 228-237.

Kunkee, R.E., 1967. Control of malolactic fermentation induced by *Leuconostoc citrovorum*. Am. J. Enol. Vitic. 18, 71-77.

Lategan, E.L., 2011. Determining of optimum irrigation schedules for drip irrigated Shiraz vineyards in the Breede River Valley. MSc thesis, Stellenbosch University, Private Bag X1, 7602 Matieland (Stellenbosch), South Africa.

Le Marrec, C., Bon, E. & Lonvaud-Funel, A., 2007. Tolerance to high osmolality of the lactic acid bacterium *Oenococcus oeni* and identification of potential osmoprotectants. Int. J. Food Microbiol. 115, 335-342.

Leske, P.A., Sas, A.N, Coulter, A.D., Stockley, C.S. & Lee, T.H., 1997. The composition of Australian grape juice: chloride, sodium and sulfate ions. Aust. J. Grape and Wine Res. 3, 26-30

London, J., 1976. The ecology and taxonomic status of the lactobacilli. Ann. Rev. Microbiol. 30, 279-301.

Lonvaud-Funel, A., 1999. Lactic acid bacteria in the quality improvement and depreciation of wine. Antonie van Leeuwenhoek 76, 317-331.

Malandra, L., Wolfaardt, G., Zietsman, A. & Viljoen-Bloom, M., 2003. Microbiology of a biological contactor for winery wastewater treatment. Water res. 37, 4125-4134.

Malherbe, S., 2007. Industry-wide assessment and characterisation of problem fermentations. MSc thesis, Stellenbosch University, Private Bag X1, 7602 Matieland (Stellenbosch), South Africa.

McCarty, P.L., 1964. Anaerobic waste treatment fundamentals. Chemistry and Microbiology. Part 1. Public Works. September.

McCarthy, M.G., 1981. Irrigation of grapevines with sewage effluent. I. Effects on yield and petiole composition. Am. J. Enol. Vitic. 32, 189-196.

Mpelasoka, B.S., Schachtman, D.R., Treeby, M.T. & Thomas, M.R., 2003. A review of potassium nutrition in grapevines with special emphasis on berry accumulation. Aust. J. Grape Wine Res. 9, 154-168.

Mulidzi, A.R., Wooldridge, J., Laker, M.C. & Van Schoor, L., 2009. Composition of effluents from wineries in the Western and Northern Cape Provinces I. Seasonal variations and differences between wineries. Wineland, January. pp. 88-91.

Myburgh, P.A., 2006. Juice and wine quality responses of *Vitis vinifera* L. *cvs. S*auvignon blanc and Chenin blanc to timing of irrigation during berry ripening in the coastal region of South Africa. S. Afr. J. Enol. Vitic. 27, 1-7.

Myburgh, P.A., 2011. Moontlike aanpassings in besproeiing en prieelstelsel om waterverbruikeffektiwiteit van wingerde te verbeter (Deel 6): Produksie, waterverbruikeffektiwiteit en gehalte van Pinotage. Wineland, June. pp. 85-87.

Neilsen, G., Stevenson, D. & Fitzpatrick, J., 1989. The effect of municipal wastewater irrigation and rate of N fertilization on petiole composition, yield and quality of Okanagan Riesling grapes. Can. J. Plant Sci. 69, 1285-1294.

Novak, P., 2002. Cineole – a new aroma component of Pinot Noir grape juice in Tasmania. Honours thesis, School of Agricultural Science, University of Tasmania.

Ojeda, H., Andary, C., Kraeva, E., Carbonneau, A. & Deloire, A., 2002. Influence of pre- and postvéraison water deficit on synthesis and concentration of skin phenolic compounds during berry growth of *Vitis vinifera* cv. Shiraz. Am. J Enol. Vitic. 53, 261-267.

Phaff, H.J., Miller, M.W. & Mrak, E.M., 1978. The life of yeasts ($2^{nd}$ ed). Harvard University Press, Cambridge.

Rankine, B.C., Fornachon, J.C.M., Boehm, E.W. & Cellier, K.M., 1971. Influence of grape variety, climate and soil on grape composition and on the composition and quality of table wines. Vitis. 10, 33-50.

Ribéreau-Gayon, P., Glories, Y., Maujean, A. & Dubourdieu, D., 2006 ($2_{nd}$ ed). Handbook of enology, volume 2. The chemistry of wine stabalization and treatments. John Wiley & Sons Ltd, Chichester.

Rodriguez, S.B., Amberg, E. & Thornton, R.J., 1990. Malolactic fermentation in Chardonnay: growth and sensory effects of commercial strains of *Leuconostoc oenos*. J. Appl. Bacteriol. 68, 139-144.

Rühl, E.H., 1989. Effect of potassium and nitrogen supply on the distribution of minerals and organic-acids and the composition of grape juice of Sultana vines. Aust. J. Exp. Agr. 29, 133-137.

Sheridan, C.M., Glasser, D., Hildebrandt, D., Petersen, J. & Rohwers, J., 2011. An annual and seasonal characterisation of winery effluent in South Africa. S. Afr. J. Enol. Vitic. 32, 1-8.

Showa Denko Europe, 2008. Analysis of anions in wine by Ion Chromatography. (http://www.showa-denko.com/index.php?id=35&tx_ttnews%5Btt_news%5D=63&tx_ttnews%5BbackPid%5D=36&cHash=49c48f7b67).

Somers, T.C., 1975. In search of quality for red wines. Food Technol. Aust. 27, 49-56.

Son, H., Hwang, G., Kim, K.M., Ahn, H., Park, W., Van Den Berg, F., Hong, Y. & Lee, C., 2009. Metabolomic studies on geographical grapes and their wines using H NMR analysis coupled with multivariate statistics. J. Agric. Food Chem. 57, 1481-1490.

South African Wine Laboratories Association, 2003. Chemical and physical analysis. Methods of analysis for wine laboratories. South African Society for Enology and Viticulture, p 1.3.1-6.1.1.

Stamer, J.R., 1979. The lactic acid bacteria: microbes of diversity. Food Technol. 33, 60-65.

Stevens, R.M. & Walker, R.R., 2002. Response of grapevines to irrigation-induced saline sodic soil conditions. Aust. J. Exp. Agric. 42, 323-331.

Stevens, R.M., Harvey, G. & Partington, D.L., 2011. Irrigation of grapevines with saline water at different growth stages: effect on leaf, wood and juice composition. Aust. J. Grape Wine Res. 17, 239-248.

Tamas, M.J. & Hohmann, S., 2003. The osmotic stress response of *Saccharomyces cerevisiae*. In: Hohmann, S. & Mager, W.H. (eds). Yeast stress responses, Springer-Verlag, Heidelberg. pp. 121-200.

Trainotti, N. & Stambuk, B., 2001. NaCl stress inhibits maltose fermentation by *Saccharomyces cerevisiae*. Biotechnol. Lett. 23, 1703-1707.

Van Leeuwen, C. & Seguin, G., 2006. The concept of terroir in viticulture. J. Wine Res. 17, 1-10.

Van Leeuwen, K., Pardon, K., Elsey, G., Sefton, M. & Capone, D., 2007. Are Australian wines affected by the proximity of vineyards to eucalypt trees? Determination of 1,8-cineole (eucalyptol) in red and white wines. Proc. 13th Aust. Wine Ind. Tech. Conf., Adelaide, Australia. pp. 389-390.

Van Schoor, L.H., 2005. Guidelines for management of wastewater and solid waste at existing wineries. Winetech, South Africa. (http://www.winetech.co.za/index.php).

Van Zyl, J.L., 1981. Waterbehoefte en besproeiing. In: Burger, J. & Deist, J. (eds). Wingerdbou in Suid-Afrika. ARC Infruitec-Nietvoorbij, Stellenbosch, South Africa. pp. 234-282.

Walker, G.M., 1998. Yeast physiology and biotechnology. John Wiley and Sons, New York.

Walker, R.R., Blackmore, D.H., Clingeleffer, P.R., Godden, P., Francis, L., Valente, P. & Robinson, E., 2003. Salinity effects on vines and wines. Bull. O.I.V. 76, 200-227.

White, R.E., 2003. Soils for Fine Wines, Oxford University Press, New York.

Williams, L.E., Dokoozlian, N.K. & Wample, R., 1994. Grape. In: Schaffer, B. & Anderson, P.C. (eds). Handbook of Environmental Physiology of Fruit Crops, Vol. I. Temperate Crops. CRC Press, Orlando. pp. 83-133.

Wibowo, D., Eschenbruch, R., Davis, C.R., Fleet, G.H. & Lee, T.H., 1985. Occurrence and growth of lactic acid bacteria in wine: A review. Am. J. Enol. Vitic. 36, 302-313.

Wibowo, D., Fleet, G.H., Lee, T.H. & Eschenbruch, R.E., 1988. Factors affecting the induction of malolactic fermentation in red wine with *Leuconostoc oenos*. J. Appl. Bacteriol. 64, 421-428.

# Chapter 5

## Effect of direct contact between berries and winery wastewater on wine sensorial characteristics

# 5. RESEARCH RESULTS

## 5.1 INTRODUCTION

Water shortage is one of the primary factors limiting production in numerous vineyards around the world (Williams *et al.,* 1994; Laurenson *et al.,* 2010). A decrease in wine quality can also be expected if water shortages arise and irrigation is not applied judiciously (Williams *et al.,* 1994). In addition to water availability, the quality of irrigation water is also of the utmost importance. Due to the plentiful amounts of readily available water, water quality aspects have been neglected in the past (Ayers and Westcot, 1985). The intensive depletion of high quality water supplies has resulted in research into alternative water supplies of poorer quality (Ayers and Westcot, 1985). Winery wastewater for irrigation purposes is being investigated as one of these supplementary water sources.

Winery wastewater contains high numbers of microorganisms, ranging from $10^5$ to $10^8$ colony forming units per millilitre (cfu/mL) (Jourjon *et al*., 2005). The dominant yeast species are *Saccharomyces cerevisiae*, *Candida intermedia, Hanseniaspora uvarum* and *Pichia membranaefaciens* (Malandra *et al.,* 2003). Winery wastewater also contains high lactic acid bacteria (LAB) and acetic acid bacteria (AAB) populations (Jourjon *et al*., 2005). Therefore, if contact is made between winery wastewater and grapes during irrigation, some microbes may survive on grape berries and end up in grape must and wine. If certain unfavourable microbes are transferred from the wastewater into the juice and wine, wine composition and quality may be detrimentally affected.

Winery wastewater has a foul smell due to the conversion of organic compounds to, among others, methane under anaerobic conditions (McCarty, 1964). If these off-odours are transferred onto or into berries and the resulting wines, it may result in tainted wines. For example, if winery wastewater irrigation is applied through overhead irrigation, contact between irrigation water and bunches would be inevitable. A Study by Kennison *et al.* (2009) showed that, when grapevines were exposed to smoke between véraison and harvest it caused a ‘smoke taint’ in the resulting wines. Similarly, wines made from grapevines which are situated nearby *Eucalyptus* tree plantations, has been found to obtain higher *Eucalyptus*-like or minty characters, which may be obtained from the trees (Novak, 2002; Van Leeuwen *et al*., 2007). If these odours are transferred from the atmosphere onto or into grapes and the resulting wines, the sharp, foul odour of winery wastewater may quite possibly be transferred onto or into grapes and wine if direct contact is made between wastewater and berries.

To our knowledge the possible effects of direct contact between grapevine bunches and winery wastewater on wine sensory characteristics have not been investigated. Therefore, the primary purpose of this study was to determine whether direct contact between grapevine bunches and winery wastewater affected wine sensorial quality in any manner. In addition, the sensorial wine-aroma analysis would primarily investigate whether off-odours excreted by winery wastewater can adhere to, or be absorbed by grapes and transferred into the produced wines, resulting in faulty wines.

## 5.2 MATERIALS AND METHODS

### 5.2.1 Experimental layout

The same vineyard, as described in Chapter 3, was used for this study. This study was performed only in the 2012 season. Drip irrigation was used instead of micro-sprinkler irrigation. Grapevine bunches were sprayed using normal water spray bottles with three different water treatments: Two different treatments with winery wastewater and a control treatment using water from the Holsloot river *ca.* 100 m from the vineyard. The two wastewater treatments used were: 50% raw winery wastewater/50% river water (3000 mg/L COD) and 100% raw winery wastewater (6000 mg/L COD). Furthermore, the foul odour intensity of the water treatments increases as the level of COD increases. Each treatment was applied to three experimental grapevines, receiving four spray treatments within three weeks prior to harvest. Treatments were applied approximately once every five days by wetting only the bunch zone. Furthermore, treatments were applied on warm, sunny days at noon. Each grapevine was considered as a treatment replication, thus each of the three treatments were replicated three times. Treatment application entailed complete water coverage of all bunches with the various water quality treatments.

### 5.2.2 Small scale vinification

Wines were made in the 2012 vintage. Grapes, harvested at *ca.* 24 degree balling (˚B), from each experimental grapevine were crushed separately by hand and allocated to 2 L plastic containers. At least one hour skin contact was allowed before inoculation with rehydrated pure wine yeast (VIN 13, Anchor Yeast, Cape Town, South Africa) at 30 g/hL. Di-ammonium phosphate (DAP) was added immediately after inoculation at 50 g/hL as yeast nutrition. Alcoholic fermentation (AF) was conducted on the skins at 25 ˚C, during which the skin caps were punched down two times a day to ensure sufficient skin contact and extraction. Must was fermented to between 0 °B and 2 °B, after which skins were separated and pressed by using a handmade steel press. Press and free run wines were combined, after which it was fermented to dryness at 25 °C. After completion of AF, 50 mg/L sulphur dioxide ($SO_2$) was added to the wines. Wines were stored at 14 °C until sensory evaluation in June 2012.

### 5.2.3 Sensorial wine quality

Wine sensorial evaluation was performed by an expert panel consisting of 11 judges. Only the visual appearance and sensorial aroma of the wines were evaluated. Wines were scored on the occurrence of off-odours and the presence of atypical colour, overall intensity, vegetative character, berry character, spicy character and overall quality. Off-odours were further evaluated within three sub categories: volatile acidity, wastewater odour and other off-odours. Wines were scored by means of a 100 mm unmarked line scale. Each score mark was measured and averages determined. Average distance was used to determine the degree of presence/liking/disliking by the judges. The average score for each treatment was calculated as an average score of the triplicates. Wines were not tasted as its absolute safety could not be guaranteed.

### 5.2.4 Statistical analysis

Microsoft® Excel (Microsoft Corporation, USA) was used to sort raw data. Data were subjected to an analysis of variance (ANOVA) by using Statistica version 10 (Statsoft, USA). Significant differences were expressed using 90% confidence intervals.

## 5.3 RESULTS AND DISCUSSION

### 5.3.1 Sensorial wine quality

Direct contact between grapevine bunches and augmented winery wastewater had no significant effect on wine colour, overall intensity, vegetative character, berry character, spicy character or overall wine quality. A clear tendency of decreased spicy character was, however, observed with an increase in the level of COD in the treatment water. This is largely inexplicable, but may have been the result of conflicting odours masking or changing the commonly known spicy nuances. Even though wine volatile acidity (VA) was not significantly increased, it showed a trend to increase as the level of COD in the winery wastewater increased.

Winery wastewater contains large populations of acetic acid bacteria (Jourjon *et al*., 2005). If these bacteria are transferred into grape juice and wine, it may cause an increase in wine VA. Juice and wine microbial populations were, however, not monitored and could not be related to these findings. Winery wastewater odour was increased in the wines as the COD concentration in the winery wastewater increased. This increase indicates that off-odours, present in winery wastewater, may possibly adhere to and/or be absorbed by berries when contact is made between wastewater and berries *via* irrigation or by other means. Although overall wine quality was not reduced by treating the grapes with wastewater in this study, the results indicate that

there may be a risk that wines may be spoiled if contact of grapes and wine with winery wastewater is not avoided. As the winery wastewater off-odour was recognisable in the wine aroma, it is possible that it may also have affected the palate. As wines were not tasted, this aspect could not be investigated further. Any other wine off-odours were, however, not affected when direct contact between grapevine bunches and winery wastewater was made.

## 5.4 CONCLUSIONS

The results of this preliminary study suggest that direct contact between grapevine bunches and winery wastewater may cause a decrease in spicy character, increase in wine VA as well as result in the presence of a winery wastewater-like off-odour in wines. Furthermore, as the quality of the water decreases, these off-odours increase. Therefore, even though wine colour and common sensory wine descriptors were not affected by the various treatments, any further increase in wine VA or wastewater off-odour may reduce wine quality. Furthermore, wastewater odours may change from winery to winery and the risk for off-flavour occurrence can therefore not be excluded.

The effect of direct contact of grapes with winery wastewater was not investigated comprehensively in this study, as only two wastewater irrigation treatments were compared to the raw water irrigation control. In addition, the study was only done during one vintage. Further investigation into the effect of direct contact between winery wastewater and grapevine bunches is therefore required before final conclusions can be made. However, this study indicated that direct contact between winery wastewater and grapes should rather be avoided in order to reduce the risk of spoiling wine sensorial quality.

**Table 5.1** Aroma analysis of wines made from grapes of Cabernet Sauvignon/99R sprayed using winery wastewater during the 2012 season.

| Treatment no. | Target COD (mg/L) | Colour | Overall intensity | Vegetative | Berry | Spicy | Volatile acidity | Wastewater odour | Other off odours | Overall quality |
|---|---|---|---|---|---|---|---|---|---|---|
| T1 | Raw water | 55.9 a[(1), (2)] ± 3.4 | 47.7 a ± 6.9 | 11.9 a ± 3.1 | 41.1 a ± 8.6 | 14.8 a ± 3.0 | 4.8 a ± 0.9 | 2.9 a ± 1.6 | 3.7 a ± 1.7 | 43.1 a ± 7.1 |
| T2 | 3000 | 72.7 a ± 7.3 | 47.7 a ± 1.9 | 12.8 a ± 5.2 | 39.3 a ± 5.8 | 12.8 a ± 5.1 | 7.5 a ± 2.6 | 5.7 ab ± 1.7 | 6.8 a ± 3.6 | 41.0 a ± 4.0 |
| T3 | 6000 (Raw wastewater) | 56.6 a ± 20.9 | 49.1 a ± 9.1 | 14.5 a ± 9.6 | 40.0 a ± 10.8 | 5.7 a ± 3.5 | 7.7 a ± 1.7 | 6.9 b ± 1.0 | 2.2 a ± 1.6 | 37.5 a ± 9.6 |

[(1)] Values designated by the same letters within a column do not differ significantly ($p \leq 0.10$).

[(2)] Values represent a score out of 100

± Values indicate standard deviation from the mean

## 5.5 LITERATURE CITED


Ayers, R.S. & Westcot, D.W., 1985. Water quality for agriculture. Food and Agriculture Organization of the United Nations, Rome.

Jourjon, F., Khaldi, S., Reveillere, M., Thibault, C., Poulard, A., Chretien, P. & Bednar, J., 2005. Microbiological characterization of winery effluent: an inventory of the sites for different treatment systems. Water Sci. Technol. 51, 19-26.

Kennison, K.R., Wilkinson, K.L., Pollnitz, A.P., Williams, H.G. & Gibberd, M.R., 2009. Effect of timing and duration of grapevine exposure to smoke on the composition and sensory properties of wine. Aust. J. Grape Wine Res. 15, 228-237.

Laurenson, S., Bolan, N., Smith, E. & McCarthy, M., 2010. Winery wastewater irrigation: effects of sodium and potassium on soil structure. CRC CARE Technical Report 19, 1-25. (http://www.crccare.com/publications/downloads/CRC-CARE-Tech-Report-19.pdf).

Malandra, L., Wolfaardt, G., Zietsman, A., & Viljoen-Bloom, M., 2003. Microbiology of a biological contactor for winery wastewater treatment. Water res. 37, 4125-4134.

McCarty, P.L., 1964. Anaerobic waste treatment fundamentals. Chemistry and Microbiology. Part I. Public Works. September.

Novak, P., 2002. Cineole – a new aroma component of Pinot Noir grape juice in Tasmania. Honours thesis, School of Agricultural Science, University of Tasmania.

Van Leeuwen, L., Pardon, G., Elsey, G., Sefton, M. & Capone, D., 2007. Are Australian wines affected by the proximity of vineyards to eucalypt trees? Determination of 1,8-cineole (eucalyptol) in red and white wines. Proceedings of 435 the 13th Australian wine industry technical conference, Adelaide, Australia.

Williams, L.E., Dokoozlian, N.K. & Wample, R., 1994. Grape. In: Schaffer, B. & Anderson, P.C. (eds). Handbook of Environmental Physiology of Fruit Crops, Vol. I. Temperate Crops. CRC Press, Orlando. pp. 83-133.

# Chapter 6

## General discussion and conclusions

# 6. GENERAL DISCUSSION AND CONCLUSIONS

## 6.1 GENERAL DISCUSSION

Grapevine growth and subsequent wine quality are affected by various factors of which the availability and quality of water for irrigation are among the most important (Ayers and Westcott, 1985; Williams *et al.,* 1994). South Africa is already a relatively dry country (Department of Water Affairs and Forestry, 2004). With an expected temperature increase in the range of 1 ˚C to 3 ˚C and a broad reduction in rainfall of between 5% and 10% for the summer rainfall region by the middle 21$^{st}$ century (Department of Environmental Affairs and Tourism, 2004), the availability of water resources will become scarcer as the country becomes increasingly dry. Furthermore, water shortage is one of the primary factors affecting production and grape quality (Williams *et al.,* 1994; Hamilton *et al*., 2005; Anderson *et al.,* 2008; Laurenson *et al.,* 2010). For these reasons, winery wastewater as an alternative source for vineyard irrigation has of late been receiving increasing attention.

Winery wastewater contains higher amounts of certain elements, such as sodium (Na) and potassium (K) (Mulidzi *et al.,* 2009; Sheridan *et al.,* 2011), which may affect soil structure and chemical composition as well as grapevine water status, growth and wine quality (Downton and Loveys, 1981; Munns and Termaat, 1986; Shannon and Grieve, 1999; Laurenson *et al.,* 2010). In addition, winery wastewater also contains large yeast and bacteria populations (Jourjon *et al*., 2005; Malandra *et al.,* 2003), which could affect wine composition and sensorial quality if they are transferred into juice and wine. Therefore, it is important that the effects of irrigation using winery wastewater on soil, grapevines and wine quality be investigated comprehensively. The overall objective of this study was to investigate the effects of irrigation with winery wastewater, augmented with raw irrigation water to different levels of COD, on grapevine response, juice and wine composition, and sensorial wine quality in the Breede River Valley. In addition, it was important to identify at what concentration the wastewater irrigation would be detrimental, if at all. To our knowledge, this is the first study that investigated the effects of irrigation using augmented winery wastewater on grapevine response, juice and wine composition, as well as sensorial wine quality.

With the exception of an increase in juice and wine pH and a decrease in wine total titratable acidity (TTA), irrigation using augmented winery wastewater within the applied ranges did not affect grapevine vegetative and reproductive growth, juice and wine chemical or microbial composition, or sensorial wine quality. Relationships between grapevine water status and vegetative and reproductive growth indicate that wastewater irrigation did not decrease the osmotic

potential of the soil solution significantly to impede water uptake, even though the irrigation water contained higher salt concentrations. Furthermore, similar vegetative growth resulted in similar grapevine microclimates. Increased juice and wine pH and decreased wine TTA indicate that grapevine physiological functioning may be affected by irrigation using winery wastewater, probably resulting from increased amounts of Na and K, which are applied through irrigation with winery wastewater at increasing concentrations as the level of COD increases. When large amounts of Na and K are applied to soils, these cations may bind to free acids in grapes and wine, resulting in salt formation from malic and tartaric acids and an associated decrease in TTA and increase in pH (Boulton, 1980; Iland and Coombe, 1988; Mpelsoka *et al.,* 2003). However, juice TTA was not affected, probably because insufficient Na and K had been extracted to bind with the free acids during normal preparation for juice analysis. Even though there was an increase in the amount of elements applied through irrigation as the level of COD in the irrigation water increased, no differences with regards to treatments were observed in the amount of elements, ions and heavy metals in the juice and wine. This indicates that grapevines did not absorb these elements, ions or heavy metals to a greater extent just because they were present in larger amounts. This further suggests that these elements, if not removed through leaching or by means of an interception crop, may accumulate in the soil and may result in phytotoxicity; under such circumstances juice and wine TTA and pH may be greatly affected.

Yeast and bacteria species were not transferred from the irrigation water onto the grapes and into the juice and wine. As contact between irrigation water and grapes would only be made when using over-head irrigation or when irrigating in very windy conditions with micro-sprinklers, the possibility of transferring harmful yeast, bacteria or pathogenic microorganisms from winery wastewater onto the grapes and into the juice and wine is probably highly unlikely. The tempo of alcoholic fermentation (AF) and malolactic fermentation (MLF), in addition to yeast and lactic acid bacteria (LAB) growth, was not affected by wastewater irrigation as the only two parameters that were affected, namely TTA and pH, was not affected to a large enough extent. Still, care should be taken that wine pH is not increased to very high values as growth of *Lactobacillus* and *Pediococcus* species may be favoured to growth of *O. oeni* at pH values greater than 3.5 (Henick-Kling, 1993). The increase in wine pH may also be considered advantages for cooler winegrowing regions as cool ripening conditions result in high acid wines with low pH values, resulting in harsh fermentation conditions for LAB to conduct MLF. Malolactic activity is also increased at higher pH values, between 3.5 and 4.0 (Bauer and Dicks, 2004). High irrigation frequencies and rapid completion of AF resulted in thin, diluted wines with regards to colour and flavour. This was apparent for wines from all nine treatments, with regards to overall wine quality as well as individual sensory attributes and off-flavours. Odours, excreted from the highest wastewater

irrigation concentrations, were not transferred into wines when irrigation was applied *via* micro-sprinklers.

When direct contact was made between berries and winery wastewater, volatile acidity (VA) and winery wastewater-like off-odours were found to be more prominent in wines made from grapes that were in contact with raw or diluted winery wastewater, when compared to the raw river water control. Unfortunately, no other analysis was performed to identify the cause of these odours. Still, overall quality and other sensory attributes were not affected. If off-odours are transferred from winery wastewater into wines, or produced as a response to wastewater irrigation, it may cause wine spoilage. Based on these limited observations, it may still be good practice to rather avoid contact between wastewater and grape berries in both vineyard and winery.

## 6.2 CONCLUSIONS

The pressure on available water resources because of urban and agricultural needs has gained momentum with the predicted climate change. This has triggered enormous focus on cleaner production, a decrease in the wine industry carbon footprint as well as preservation of available water resources and water re-use. Preliminary results obtained from this study indicate that irrigation using augmented winery wastewater may contribute to decreasing of the carbon footprint of the wine industry, while relieving some of the pressure on water resources, without severely impacting on any viticulture or wine quality aspects.

It is of utmost importance to take into consideration that this study was conducted over a two year period under specific soil and climatic conditions, using wastewater generated from one cellar, and irrigated at a limited range of concentrations. It is possible that elements, and therefore the effect of irrigation using augmented winery wastewater, may accumulate over time and only start impacting on soil, grapevine and wine quality after a longer period of time. Therefore, it is recommended that an interception crop, with the ability to absorb large amounts of Na and K, be planted when irrigating winery wastewater onto grapevines. The interception crop would thus serve the purpose of avoiding the build-up of Na and K in the soil. As this is an ongoing study, it is recommended that results obtained from the next two seasons are also taken into account before final conclusions are made with regards to the effect of wastewater irrigation under the given conditions. In addition, if a different rootstock or cultivar is used under different climatic and field conditions, entirely different results may be obtained. Heavier soils may be affected more severely and rapidly, resulting in greater effects on grapevines. Furthermore, irrigation using more concentrated winery wastewater may also have a greater effect. The data gathered and knowledge obtained from this thesis made a valuable contribution to understanding the effects of irrigation using augmented winery wastewater and its possible future use for vineyard irrigation. Still, much more research would be

required to better understand the long term effect of irrigation using augmented winery wastewater under different field conditions and with a wider range of parameters.

## 6.3 RECOMMENDATIONS FOR FUTURE RESEARCH

Even though the effects of irrigation using augmented winery wastewater were investigated broadly and meticulously in this study, various research possibilities still exist and arose from this study. These include:

- Applying irrigation treatments using more concentrated/less diluted winery wastewater to determine whether a more rapid and greater response is obtained. Due to the fact that few irrigation treatment effects were observed in this study, the effect of higher wastewater concentrations should be investigated to determine at what concentration grapevines response as well as juice and wine composition is affected considerably.
- Evaluating the effect of irrigation using augmented winery wastewater on different cultivars and soil under different climatic conditions. The osmotic tolerance and extent to which elements are absorbed by grapevines are largely variable between different rootstocks and soils. If larger amounts of elements are absorbed, more rapid and more extensive results may be obtained. Furthermore, heavier soils may deteriorate faster than the sandy soils used in this study, causing more rapid responses.
- Conducting a study where no interception crop is planted, as it may interfere with results by extracting certain elements, masking or decreasing their possible impact on the main parameters measured.
- Monitoring acetaldehyde and glycerol production in wines, as their production by the yeast is stimulated when yeast cells experience osmotic stress. Osmotic stress may result from irrigation using winery wastewater if NaCl levels in juice and wine are increased.
- Generating analytical data regarding the aroma compounds in wine to assess whether wastewater irrigation has an impact on their presence. When performing sensorial wine evaluations, subtle impacts on flavour compounds may not be noticed, as the human sensory detection thresholds are higher than the analytical detection thresholds.
- Determining the compounds in winery wastewater which are responsible for the foul odour it releases and determine whether these compounds are present in wines made from grapevines that received irrigation using augmented winery wastewater.
- Specifically identify all yeast and bacteria strains present in winery wastewater and in juice and wine made from grapevines receiving wastewater irrigation, to assess whether microbes are transferred from the irrigation water to the juice and wine, as well as to determine what their contributions to the wine compositional and sensory profiles are.

## 6.4 LITERATURE CITED


Anderson, K.C., Findlay, C., Fuentes, S. & Tyerman, S., 2008. Viticulture, wine and climate change. Garnaut Climate Change Review, Adelaide, Australia. pp. 1-22. (www.garnautreview.org.au).

Ayers, R.S. & Westcott, D.W., 1985. Water quality for agriculture, FAO Irrigation and Drainage Paper No. 29, FAO, Rome. pp. 1-96. (http://www.calwater.ca.gov/Admin_Record/C-110101.pdf).

Bauer, R. & Dicks, L.M.T., 2004. Control of malolactic fermentation in wine. A review. S. Afr. J. Enol. Vitic. 25, 74-88.

Boulton, R., 1980. The general relationship between potassium, sodium and pH in grape juice and wine. Am. J. Enol. Vitic. 31, 182-186.

Department of Environmental Affairs and Tourism, 2004. A national climate change response strategy for South Africa. *(*unfccc.int/files/meetings/seminar/.../pdf/sem_sup3_south_africa.pdf).

Department of Water Affairs and Forestry, 2004. Revision of general authorisation in terms of Section 39 of the National Water Act, 1998 (Act 36 of 1998). Section 21, Government Notice 1091, Government Gazette 26187, 13, 1-33. August 1998, Department of Water Affairs and Forestry, Pretoria, South Africa.

Downton, W.J.S. & Loveys, B.R., 1981. Abscisic acid content and osmotic relations of salt-stressed grapevine leaves. Aust. J. Plant Physiol. 8, 443-452.

Hamilton, A.J., Boland, A.M., Stevens, D., Kelly, J., Radcliffe, J., Ziehrl, A., Dillon, P. & Paulin, B., 2005. Position of the Australian horticultural industry with respect to the use of reclaimed water. Agr. Water Manage. 71, 181-209.

Henick-Kling, T., 1993. Malolactic fermentation. In: Fleet, G.H. (ed.). Wine Microbiology and Biotechnology, Harwood Academic Publishers, Chur, Switzerland, pp. 289-326.

Iland, P.G. & Coombe, B.G., 1988. Malate, tartrate, potassium and sodium in flesh and skin of Shiraz grapes during ripening: concentration and compartementation. Am. J. Enol. Vitic. 39, 71-76.

Jourjon, F., Khaldi, S., Reveillere, M., Thibault, C., Poulard, A., Chretien, P. & Bednar, J., 2005. Microbiological characterization of winery effluent: an inventory of the sites for different treatment systems. Water Sci. Technol. 51, 19-26.

Laurenson, S., Bolan, N., Smith, E. & McCarthy, M., 2010. Winery wastewater irrigation: effects of sodium and potassium on soil structure. CRC CARE Technical Report 19, 1-25.
(http://www.crccare.com/publications/downloads/CRC-CARE-Tech-Report-19.pdf).

Malandra, L., Wolfaardt, G., Zietsman, A., & Viljoen-Bloom, M., 2003. Microbiology of a biological contactor for winery wastewater treatment. Water res. 37, 4125-4134.

Mpelasoka, B.S., Schachtman, D.R., Treeby, M.T. & Thomas, M.R., 2003. A review of potassium nutrition in grapevines with special emphasis on berry accumulation. Aust. J. Grape Wine Res. 9, 154-168.

Mulidzi, A.R., Wooldridge, J., Laker, M.C. & Van Schoor, L., 2009. Composition of effluents from wineries in the Western and Northern Cape Provinces I. Seasonal variations and differences between wineries. Wineland, January. pp. 88-91.

Munns, R. & Termaat, A., 1986. Whole-plant responses to salinity. Aust. J. Plant Physiol. 13, 143-160.

Shannon, M.C. & Grieve, C.M., 1999. Tolerance of vegetable crops to salinity. Sci. Hortic. 78, 5-38.

Sheridan, C.M., Glasser, D., Hildebrandt, D., Petersen, J. & Rohwers, J., 2011. An annual and seasonal characterisation of winery effluent in South Africa. S. Afr. J. Enol. Vitic. 32, 1-8.

Williams, L.E., Dokoozlian, N.K. & Wample, R., 1994. Grape. In: Schaffer, B. & Anderson, P.C. (eds). Handbook of Environmental Physiology of Fruit Crops, Vol. I. Temperate Crops. CRC Press, Orlando. pp. 83-133.

# Addendum A

## Microbial flora of irrigation water and must

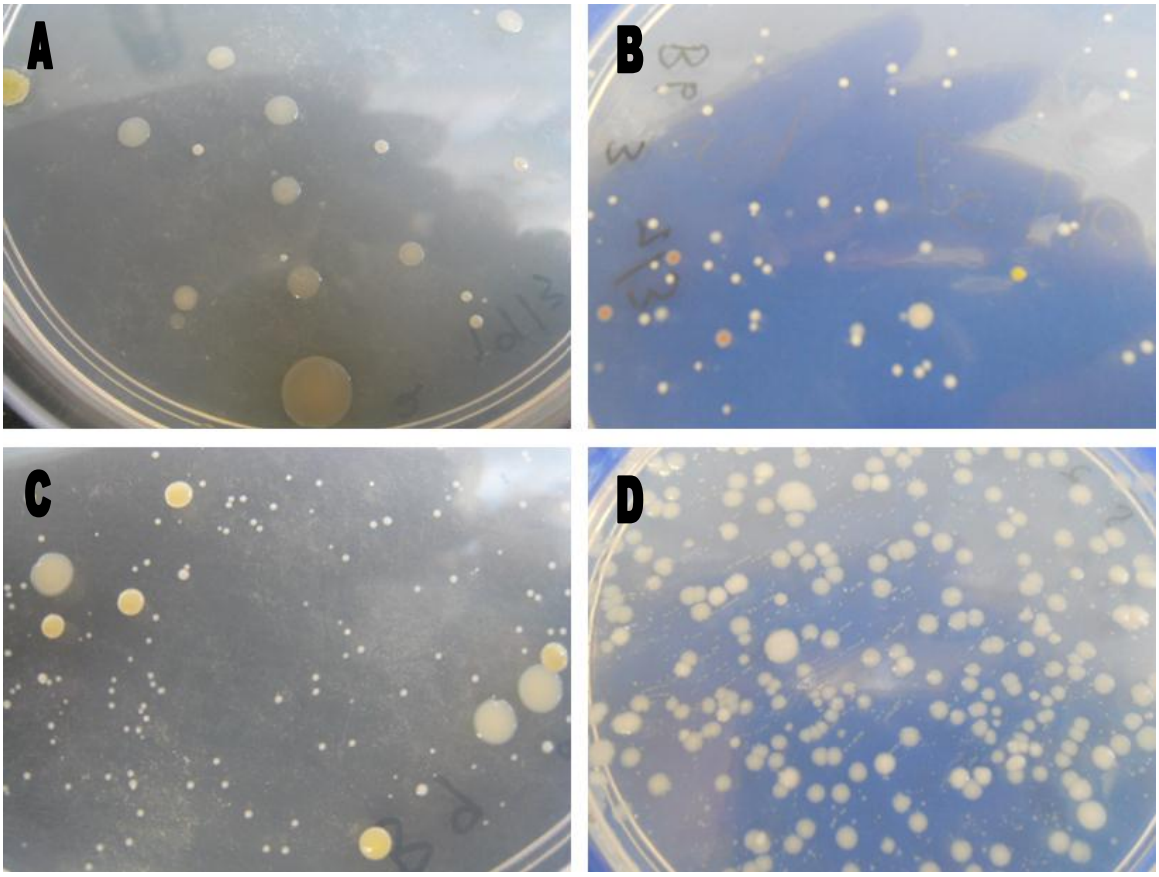


**Fig A1** Increasing numbers of microbial flora found on yeast extract agar plates from selected irrigation water treatments and raw winery wastewater. (A) T1 - Raw river water; (B) T6 - 1500 COD; (C) T9 - 3000 COD; (D) Raw winery wastewater - 7000 COD.

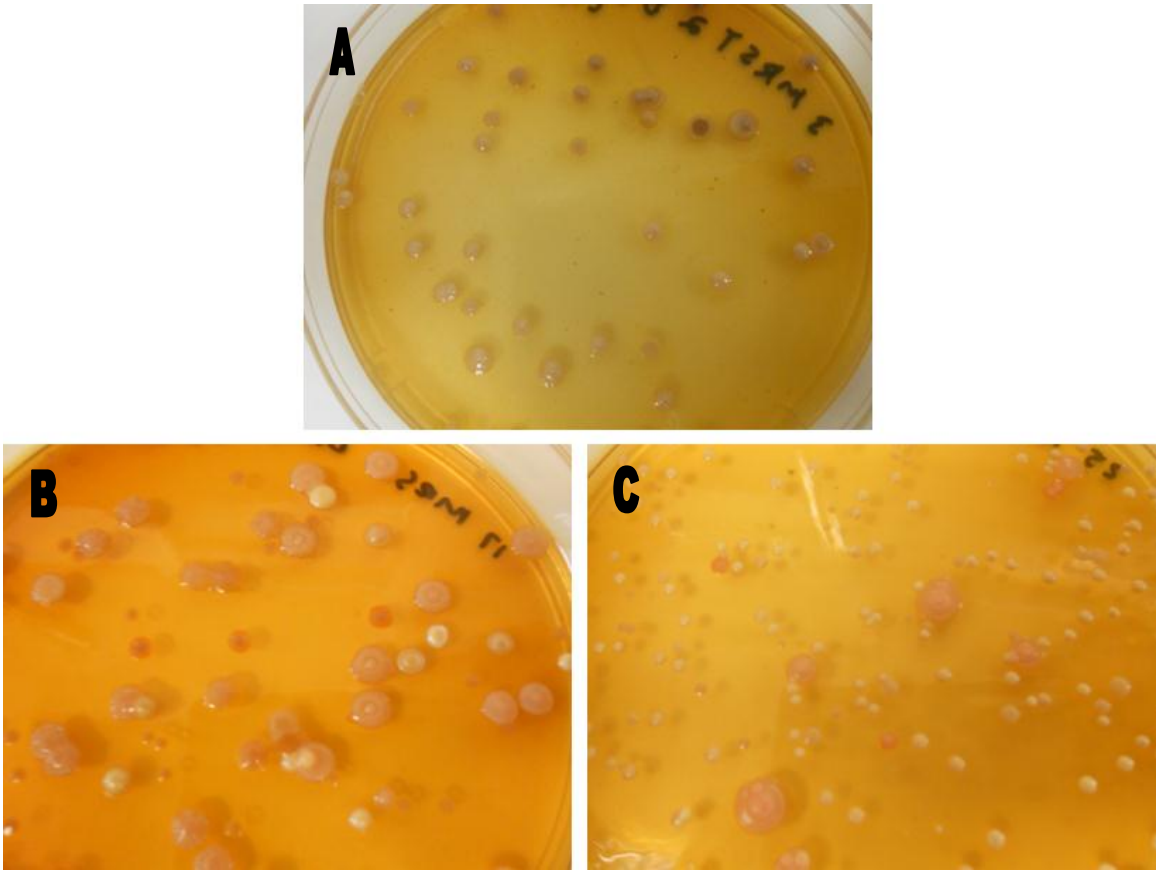


**Fig A2** LAB flora found on MRST agar plates from must from grapes of Cabernet Sauvignon/99R grapevines, irrigated using augmented winery wastewater. (A) T1 - Raw water; (B) T6 - 1500 COD; (C) T9 - 3000 COD.

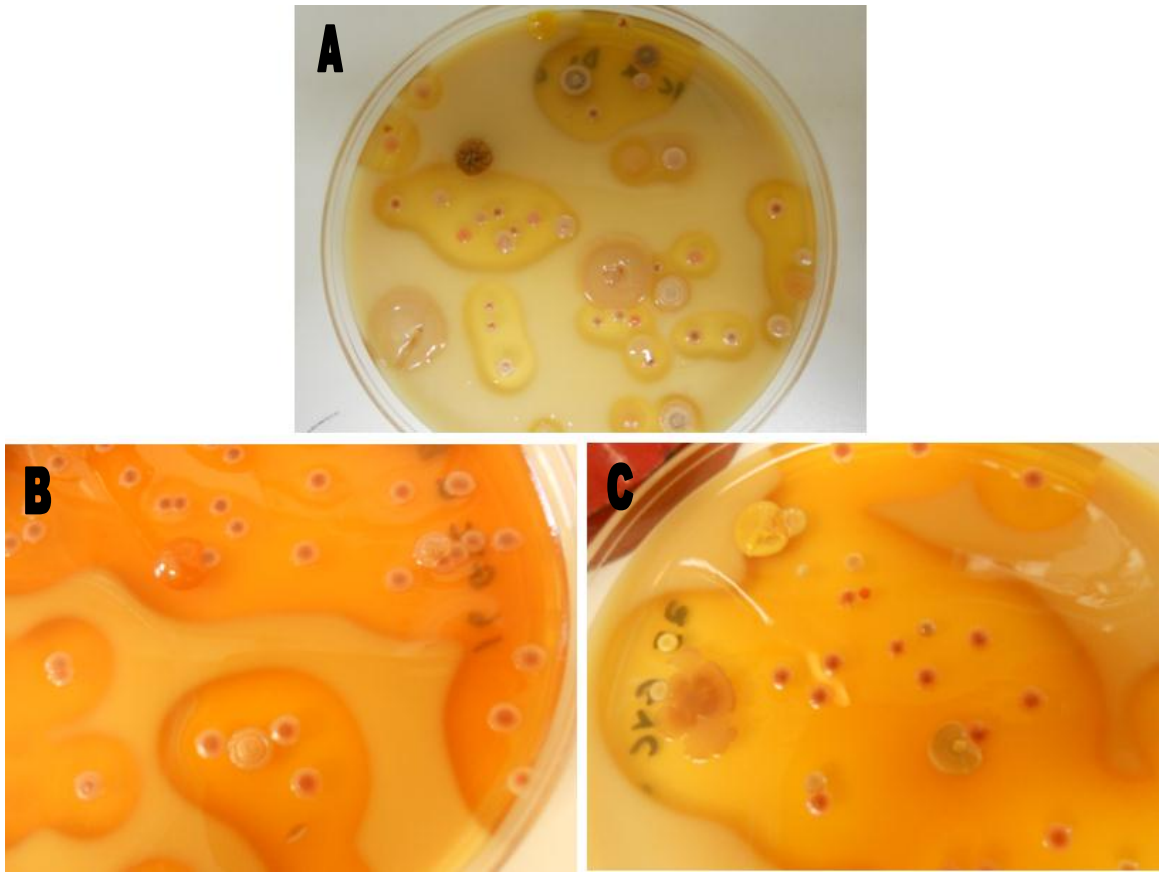


**Fig A3** Total bacteria flora found on GYC agar plates from must from grapes of Cabernet Sauvignon/99R grapevines, irrigated using augmented winery wastewater. (A) T1 - Raw water; (B) T6 - 1500 COD; (C) T9 - 3000 COD.